Citation for final published version:

Hoshino, Satoru, Seino, Satoru, Azumano, Akinori, Tuuga, Augustine, Nathan, Senthilvel K. S. S., Ramirez Saldivar, Diana A., Goossens, Benoit , Salgado-Lynn, Milens, Matsuda, Ikki and Yayota, Masato 2023. Modifying the diets of captive proboscis monkeys in a temperate zoo to reduce weight loss and renal disease. Primates 64 , pp. 123-141. 10.1007/s10329-022-01031-y

Publishers page: http://dx.doi.org/10.1007/s10329-022-01031-y

Original research paper

Title:

Modifying the diets of captive proboscis monkeys in a temperate zoo to reduce weight loss and renal disease

Authors:

Satoru Hoshino[1,2], Satoru Seino[3], Akinori Azumano[4], Augustine Tuuga[5], Senthilvel K. S. S. Nathan[5], Diana A. Ramirez Saldivar[5,6#], Benoit Goossens[5,6,7], Milena Salgado-Lynn[6,7,8], Ikki Matsuda[9,10,11,12] Masato Yayota[2,13]

Author affiliation:

1 The United Graduate School of Agricultural Science, Gifu University, Gifu 501-1193, Japan
2 Zoo Biology Research Center, Gifu University, Gifu 501-1193, Japan
3 Preservation and Research Center, Yokohama 241-0804, Japan
4 ZOORASIA, Yokohama Zoological Gardens, Yokohama 241-0001, Japan
5 Sabah Wildlife Department, Wisma Muis, 88100 Kota Kinabalu, Sabah, Malaysia
6 Danau Girang Field Centre, c/o Sabah Wildlife Department, Wisma Muis, 88100 Kota Kinabalu, Sabah, Malaysia
7 Organisms and Environment Division, Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3AX, UK
8 Wildlife Health, Genetic and Forensic Laboratory, Sabah Wildlife Department, Wisma Muis, 88100 Kota Kinabalu, Sabah, Malaysia
9 Chubu University Academy of Emerging Sciences, 1200, Matsumoto-cho, Kasugai-shi, Aichi 487-8501, Japan
10 Wildlife Research Center of Kyoto University, 2-24 Tanaka-Sekiden-cho, Sakyo, Kyoto, 606-8203

Japan
11 Borneo Conservation Trust Japan, Shinagawa, Tokyo, 140-0002, Japan
12 Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
13 Faculty of Applied Biological Science, Gifu University, Gifu 501-1192, Japan

ORCID:
Satoru Hoshino – 0000-0002-3306-1075
Benoit Goossens – 0000-0003-2360-4643
Ikki Matsuda – 0000-0002-0861-7801
Masato Yayota – 0000-0001-8056-1315
Milena Salgado-Lynn - 0000-0003-1769-6465

Author contribution

SH, IM and MY developed the initial concept; AA performed necropsies of zoo individuals; SH conducted *in situ* sample collection and analyses; SKSSN, DARS and IM conducted sample collection in the field; BG and MSL analyzed the blood samples from free-ranging individuals; SS, AT, BG and MSL arranged the sampling from the zoo and free-ranging animals; SH, IM and MY performed and interpreted the statistical analysis and drafted the manuscript; MY organized the project. All authors contributed to the final version of the manuscript.

[#] Diana A. Ramirez Saldivar is deceased.

[*]Corresponding author:
Masato Yayota
Present/permanent address: Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido,

Gifu 501-1193, Japan.
E-mail address: yayo@gifu-u.ac.jp

Short title:

Modifying diets of proboscis monkeys

Keywords:

Colobines; foregut fermenter; necropsies; hypercalcemia; renal disease; Ca/P ratio

Abstract

In animal husbandry, diets should help maintaining a healthy body condition, support reproduction and promote species-specific longevity. It is recommended to feed folivorous primates kept in zoos a high-fiber diet, i.e., leaves, although satisfying such requirement is challenging in temperate regions because it is difficult to obtain fresh leaves, especially in autumn and winter. As equally important for their appropriate treatment, it is valuable to provide details of clinical reports of medical problems and pathological findings, although such clinical reports are rather limited. Therefore, in foregut-fermenting proboscis monkeys (*Nasalis larvatus*), we 1) described the individual clinical reports of renal disease and weight loss at the Yokohama Zoological Gardens in Japan, 2) determined the nutritional profile of the diets supplied to these animals because other potential triggers for their renal disease and weight loss could be excluded, 3) modified the diet regimen to minimize weight loss and the development of hypercalcemia and hypophosphatemia, and 4) assessed the effects of such dietary modification by comparing the body weight and the Ca and P concentrations and the Ca/P ratios in the blood before and after diet modification with a comparison of these measurements between zoo and free-ranging individuals. Based on the nutritional profile of the diets, we concluded that the reported cases of renal failure might be caused by consumption of leaves with a Ca/P ratio far above the appropriate level in autumn and winter. Additionally, the dietary modification of minerals and metabolizable energy achieved certain beneficial effects on zoo-kept proboscis monkeys.

Introduction

In recent years, more than half of the world's free-ranging primate species have been threatened with extinction (Estrada et al. 2017), clearly indicating that not all species can be efficiently preserved in their natural habitats. Therefore, not only *in situ* but also *ex situ* management processes have become more important to mitigate such ongoing threats exacerbated by various human activities. Indeed, continuous efforts in zoo animal husbandry have improved animal reproductive success and welfare in zoos (Hosey et al. 2013).

Diet quality is often related to body maintenance, reproductive success and longevity (Kleiman et al. 2010). Therefore, a nutritionally optimized and balanced diet could contribute to the success of zoo animal husbandry. In particular, diets for captive foregut-fermenting primates (colobines) have been carefully selected as, historically, these animals have had shorter lifespans than free-ranging individuals and have been difficult to maintain healthy (e.g. Collins & Roberts, 1978; Hill, 1964; Hollihn, 1973). The reason for this disparity is that free-ranging wild colobine monkeys are rather folivorous in general (Matsuda et al. 2022), whereas captive colobines have often been fed diets similar to those that are fed to frugivorous and/or omnivorous primates (Hollihn 1973; Watkins et al. 1985; Edwards et al. 1997). This may have contributed to the high incidence of gastrointestinal disorders among captive colobines, as their diets may have been insufficiently fibrous or excessively digestible (Hollihn 1973; Nijboer and Clauss 2006; Clauss and Dierenfeld 2008), given that commercial fruits typically have higher nutrient density compared to wild fruits (Nijboer and Dierenfeld 1996; NRC 2003; Schwitzer et al. 2009). Although feeding more fibrous foods, i.e., leaves, is encouraged in captive colobines (Matsuda et al. 2018), it is often difficult to meet this requirement due to logistical constraints. Providing fresh leaves is difficult, especially during autumn and winter in temperate regions, and transporting the ideal leaves from the tropical regions that most colobines inhabit to zoos located in temperate regions is not cost-efficient. Thus, exploring nutritionally appropriate diets that have a stable supply in temperate regions is a crucial task for improving the breeding management of captive colobines in temperate regions.

It is equally important in zoo animal husbandry, especially in clinical and pathology services, to make clinical reports of medical problems and pathological findings. In colobines, however, such clinical reports, particularly on noninfectious diseases, are limited, although some notable reports describing intestinal obstruction and perforation caused by plant consumption are available (e.g. Calle et al., 1995.; Ensley et al., 1982; Janssen, 1994). Pathological findings in colobines are often centered on gastrointestinal disorders related to infectious diseases (Loomis et al. 1983; Palmieri et al. 1984; Pang et al. 1993; Overskei et al. 1994; Nishiura et al. 2019), probably due to their distinct digestive system, i.e., foregut fermentation. However, Janssen (1994) and Shelmidine et al. (2013) reported other pathological findings based on 45 necropsies in douc langurs (*Pygathrix nemaeus*) and 77 necropsies in silvered langurs (*Trachypithecus cristatus*), respectively, indicating various types of morbidity in captive colobines. Another exception is a recent study by Baker et al. (2022), reporting on three colobine species (a total of 21 cases), with a particular focus on urolithiasis-related morbidity and mortality, noting the requirement for further research into aetiology and preventive medicine. Thus, to provide appropriate medical treatment for debilitated animals and to prevent disease in captive colobines, such relevant information should be proactively shared among zoos worldwide.

Proboscis monkeys (*Nasalis larvatus*) are endemic to the island of Borneo. They consume leaves, fruits, and flowers in various proportions, although leaves generally dominate their diet (Yeager 1989; Boonratana 2003; Matsuda et al. 2009). Even compared to other colobines, these monkeys are notoriously difficult to maintain and breed in captivity, probably because of the higher intake capacity of their foregut. In captivity, the ingestion of more digestible diets compared to those eaten in the wild might lead to malfermentation (Matsuda et al. 2019; Hoshino et al. 2021). Several zoos in temperate regions have attempted to breed proboscis monkeys in captivity (Hollihn 1973; Dierenfeld et al. 1992; Nijboer and Dierenfeld 1996), but the Yokohama Zoological Gardens (YZG), Japan, is the only zoo in temperate regions that currently holds them (Ogata and Seino 2015; Inoue et al. 2016).

At YZG, eight individuals were raised from 2009 to 2018: two males and three females came from the Surabaya Zoo, Indonesia, and three females were born at YZG (Appendix F1). According to the clinical records at the zoo, however, all individuals often lost body weight in winter and suffered from constant hypercalcemia and hypophosphatemia, which are likely to trigger the development of kidney stones (Fig 1; see also Azumano et al., 2015). As a result, until June 2018, four individuals died due to renal failure.

In the present study, we first sought to describe the individual clinical reports of renal disease in captive proboscis monkeys at YZG. As detailed reports on renal disease in colobines have been rare thus far [but see Janssen (1994) and Baker et al. (2022)], our report contributes to the improvement of medical treatment for captive colobines. Based on the previous clinical reports, potential triggers of hypercalcemia and hypophosphatemia in general, such as neoplasia and parathyroid abnormalities, could be excluded (Appendix 3: see also Azumano et al., 2015). In addition, the animals were not exposed to vitamin D supplements or artificial UV radiation (but housed in the enclosure with sunlight), potentially inducing hypercalcemia. Given that urolithiasis in domestic ruminants is considered to be primarily diet-related, with strong indications that the calcium (Ca)-phosphorus (P) ratio is important (reviewed by Baker et al. 2022), dietary effects on the present case in proboscis monkeys causing hypercalcemia and hypophosphatemia, and consequently renal failure, were by contrast not ruled out as a major factor in the development of the diseases. Thus, secondly, we determined the chemical/mineral compositions of the leaves fed to the animals to verify their appropriateness as dietary items in terms of chemical/mineral composition and energy content. Nutritional analysis of their captive diet focused on fiber as it is considered to be a key nutrient for maintaining captive proboscis monkeys in healthy condition (Matsuda et al. 2018), although in free-ranging populations, protein is also considered to be important for their dietary choice (Yeager 1989; Boonratana 2003; Matsuda et al. 2017). The Ca and P concentrations and Ca/P ratio in leaves were also measured to investigate the relationship between hypercalcemia and/or hypophosphatemia, a frequent lifethreatening condition at YZG, because uncertain Ca and P concentration in leaves often complicates a diet formulation; note that the ratio of Ca to P, each of which modulates the absorption of the other, is commonly recommended to be maintained in the range of 1-2 (NRC 2003, 2007). Thirdly, based on the results of the nutritional analysis, we modified the diet regimen (feed composition and amounts) in an attempt to minimize weight loss and the development of hypercalcemia and hypophosphatemia. We assessed the effects of dietary modification by measuring weight change and blood Ca and P concentrations. In addition, to evaluate whether the values for Ca and P were in the normal range, we used values of free-ranging proboscis monkeys as reference values. Finally, we propose an appropriate feeding management system for proboscis monkeys kept in temperate regions.

Methods

Ethics statement

The data collection for the zoo animals was approved by both YZG and the Committee for Animal Research and Welfare of Gifu University (#17092). All animal experimental procedures were conducted in accordance with the Guidelines for Proper Conduct of Animal Experiments, Science Council of Japan (2006), and the guidelines on animal research and welfare of Gifu University (2008). Body weight measurement and blood analysis were conducted as part of regular health examinations at YZG. All animal handling in the wild was carried out in accordance with the current laws of Malaysia and Sabah Wildlife Department's Standard Operation Procedures on Animal Capture, Anesthesia and Welfare. Permission to collect samples from free-ranging proboscis monkeys was granted by the Sabah Wildlife Department and the Sabah Biodiversity Council. Additionally, all sampling from wild animals was in accordance with the Weatherall report (Weatherall 2006) and followed the guidelines for nonhuman primates as described by Unwin et al. (2011). All efforts were made to ensure the welfare of the animals and reduce their stress. Full personal protective equipment was worn by all team members throughout the process to prevent interspecies disease transmission.

***Ex situ* study**

Study animals and diets

Starting in 2017, measurements were performed in four proboscis monkeys at YZG: one adult male (Genki), one adult female (Kinanty) and two juveniles (Emi and Jasmine) (Appendix F1). Genki was usually housed individually, and the other three were housed together. The animals were fed a mixed diet of fresh leaves, green produce, fruits, peanuts and pellets two times daily at 09:30 and 15:00; the animals were generally moved from a nonpublic overnight enclosure to a subpaddock or the outdoor exhibition areas for their morning feeding at 09:30; later, they were moved back for their afternoon feeding at 15:00.

Leaf sampling and nutritional analysis

To analyze the nutrient levels of leaves that were consumed by the study animals, we collected the nine primary species of browse available to them: evergreen species, including bamboo-leaf oak (*Quercus myrsinifolia*), chinquapin (*Castanopsis sieboldii*), glossy privet (*Ligustrum lucidum*), laurel (*Machilus thunbergii*) and Japanese spindle tree (*Euonymus japonicus*); deciduous species, including Yoshino cherry (*Prunus yedoensis*) and willow (*Salix* spp.); tropical species, including hibiscus (*Hibiscus* spp.) and leucaena (*Leucaena leucocephala*). Bamboo-leaf oak, chinquapin, glossy privet, laurel, Yoshino cherry and willow were collected in May 2017, and Japanese spindle, hibiscus and leucaena were collected from late October to early November 2017 (Appendix T1). During each sampling period, we collected 50 g of leaf samples from the tree branches 1-3 times before feeding. Leaves, including leaf blades and leafstalks, were collected randomly from each branch. Leaves of bamboo-leaf oak, chinquapin, glossy privet and laurel were categorized into two or three maturity stages (Appendix T1). All samples were put into plastic bags and stored at -20 °C.

Leaf samples were freeze-dried for 48 h and ground with a Wiley mill to pass through a 1-

mm sieve for chemical analysis. The nutritional components were analyzed by the Agricultural Chemical Laboratory of the Tokachi Agricultural Cooperative Union (Obihiro, Hokkaido) using standard procedures, as follows. Dry matter (DM; method 930.15; AOAC (2005)), crude protein (CP; method 990.03; AOAC (2005)), ether extract (EE; method 2003.05; AOAC (2005)), and crude ash (CA; method 942.05; AOAC (2005)) were measured. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) expressed exclusively as residual ash (NDF and ADF on an organic matter basis, NDFom and ADFom, respectively) which were determined as described by Van Soest et al. (1991), as was acid detergent lignin (ADL). Neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) were determined by analyzing NDF and ADF residues for Kjeldahl nitrogen (Licitra et al. 1996). Ca, P, Mg and K contents were determined using atomic absorption spectrometry. Non-fiber carbohydrate (NFC) was calculated using the following formula: NFC = 100-CP-EE-CA-(NDFom-NDICP). Metabolizable energy (ME, kcal/g DM) was calculated using the Atwater system (Atwater, 1910): ME = (CP*4+EE*9+NFC*4)/100, with nutrient levels in % DM.

ME, Ca and P content of commercial feeds (all feedstuffs other than tree leaves) were taken from standard tables of food composition for humans (Kagawa 2005) because all these feedstuffs were produced for the purpose of human consumption. We calculated total ME, Ca and P content in the last ten diet regimens.

Dietary modification

Since renal disease had previously been observed in four individuals and weight loss (especially in autumn and winter) and hypercalcemia and hypophosphatemia in all individuals had become an issue for breeding of the study animals, we calculated total ME, Ca and P contents in seven diet regimens, not including the leaves, fed from June 2016, and we attempted to control the energy content and Ca/P ratio of the diet by changing the components other than browses three times from July to October 2017. Note that the diet regimens in the present study are represented by the measured diet composition for one individual, Genki (Appendix T2a). Specifically, we modified the amounts of foods containing more P than Ca based on the standard tables of food composition (Kagawa 2005); this modification entailed feeding more broccoli (Ca: 38 mg/100 g fresh matter [FM], P: 89 mg/100 g FM), asparagus (Ca: 19 mg/100 g FM, P: 60 mg/100 g FM), soybeans (Ca: 70 mg/100 g FM, P: 190 mg/100 g FM) and peanuts (Ca: 50 mg/100 g FM, P: 380 mg/100 g FM) but reducing the quantities of green beans, cucumbers and apples (Appendix T2b). Additionally, we modified the amounts of the foods with high energy content according to Kagawa (2005), feeding more soybeans (215.6 kcal/100 g FM) and peanuts (668.36 kcal/100 g FM) (Appendix T2b). We calculated total ME, Ca and P contents in the diet without leaves because we fed the leaves attached with twigs and branches and determining the feeding amount of only leaves was difficult.

Body weight measurement

Body weight was measured approximately once a week in the overnight enclosure. A digital weight scale (DP-8100, Yamato Co Ltd., Japan) was set on the feeding table attached to the enclosure fence, and a zookeeper guided the monkeys to the weight scale using food.

Blood sampling and analysis

To compare the effects of dietary change, blood samples were collected from each of two adult individuals (Genki and Kinanty) ten times before and three times after the dietary modifications. While the adult male (Genki) was anesthetized with 2 mg/kg ketamine and 70 μg/kg medetomidine for a routine veterinary medical examination at YZG, 3 mL of blood was collected in an EDTA-containing tube. Samples were collected from the adult female (Kinanty) while the animal was manually restrained. Immediately after blood was collected, the blood samples were centrifuged at 3,000 rpm for 10 min at room temperature (ACNO-3: FUJIFILM Medical Co., Ltd.), and the resulting plasma was used to analyze plasma Ca and inorganic phosphorus (P) content by a dry chemistry method (DRI-

CHEM400V, FUJIFILM Co. Ltd., Tokyo, Japan).

In situ study

Blood sampling and analysis

Between July 2011 and December 2016, we captured 46 free-ranging proboscis monkeys (nine adult males, ten adult females, eight subadult males, five subadult females, four juvenile males, one juvenile female, one infant male, one male of unknown age, two females of unknown age, and five individuals of unknown age and sex) in Sabah, Malaysian Borneo (5° 18′ N to 5° 42′ N and 117° 54′ E to 18° 33′ E). Of 46 individuals, seven individuals were free-ranging, provisioned individuals inhabiting a mangrove forest (ca. 260 ha) that was mostly surrounded by oil palm plantations in the Labuk Bay Proboscis Monkey Sanctuary (Hayakawa et al. 2018). To reduce the impact of capture on the animals' social system, we captured all study subjects during the night (Matsuda et al. 2020). While the animals were anesthetized, 2 mL of blood was collected in an EDTA-containing tube and kept at -80°C in the freezer for several years until processing. The blood samples were analyzed in the same way as for zoo individuals.

Data analysis

To evaluate the change in Ca and P concentrations in blood samples from captive proboscis monkeys at YZG before diet modification, we combined the data of Ca and P concentrations in blood samples collected from two individuals (Genki and Kinanty) on different days and compared the Ca and P levels and Ca/P ratio between summer (from April to October, n = 9 from two individuals) and winter (from November to March, n = 11 from two individuals) using a Wilcoxon–Mann–Whitney test. Likewise, to evaluate the effects of dietary modification on Ca and P concentrations, we combined and compared the Ca and P levels and Ca/P ratio before (n = 20 from two individuals) and after (n = 6 from two individuals) dietary modifications using a Wilcoxon–Mann–Whitney test. We also compared those values between zoo and free-ranging proboscis monkeys to evaluate whether the values for Ca and P at YZG were in the normal range. First, the Ca and P concentrations and Ca/P ratio of zoo animals before dietary modification were compared with those of free-ranging individuals living in each different habitat condition, i.e., natural or provisioned conditions, using a Wilcoxon–Mann–Whitney test with Bonferroni correction of p values (0.05/N, $N = 3$, i.e., statistical significance was accepted at $p < 0.017$). Second, the values from zoo animals after dietary modification were also compared with those from the free-ranging individuals using the same statistical approach. All statistical analyses were performed in Spyder (Python 3.7).

Results

Clinical presentation

Necropsies were performed on four proboscis monkeys, i.e., "Kurupon" (infant female), "Jaka" (adult male), "Niko" (juvenile male) and "Apuru" (adult female), at YZG (see details in Appendix T3). In Kuropon, the pathological anatomy of urinary organs confirmed the development of kidney stones in the left and right renal pelvis and the left ureterovesical junction (Fig 2a). In Jaka, kidney stones extended from the left renal pelvis to the bladder (Fig 2b), whereas the right kidney had a damaged parenchyma but no kidney stones. The left kidney of Jaka was also inflamed. In Niko, kidney stones were lodged in both the left renal pelvis and the opening of the right ureter (Fig 2c; see also Azumano et al., 2015). The left ureter of Niko was dilated, and, although no kidney stone was found at the opening of the left ureter, the left ureterovesical junction was congested and swollen (Azumano et al. 2015). The right kidney of Apuru was inflamed, and kidney stones were found in the right renal pelvis and right proximal ureter (Fig 2d). The left kidney of Apuru did not contain kidney stones but showed irregularity in shape, and the ureter was found to be dilated. Similar to the symptoms observed before death in Niko, such as weight loss, reduced food intake, anemia, hypophosphatemia and hypercalcemia (Azumano et al. 2015), marked weight loss was also observed in the other three individuals (Fig. 1).

Lastly, of all specimens, there was no evidence of neoplasia or parathyroid abnormalities, which potentially trigger hypercalcaemia or hypophosphataemia.

Chemical properties of leaves in the diet

Based on the quantity of leftovers and the ad libitum observations of foraging behavior in two adult proboscis monkeys (Kinanty and Genki) at YZG, we found that they had certain preferences for specific feedstuffs. For example, the mature leaves of bamboo-leaf oak, chinquapin, laurel, glossy privet and Japanese spindle tree were consumed markedly less than the leaves of other plant species. The chemical composition of the provided plant leaves showed that the preferred leaves (the sprouts of bamboo-leaf oak; the young leaves of bamboo-leaf oak, chinquapin, laurel and glossy privet; and the mature leaves of leucaena and Hibiscus) contained 10-20% higher CP and 5-25% lower fiber (NDFom) than the other leaves (Fig. 3).

The detailed nutritional composition in the leaves of each plant species, i.e., CP, fiber (NDFom, ADFom, ADL), NFC and minerals (Ca and P), was as follows (details available in Appendix T4). The CP contents of deciduous (Yoshino cherry and willow) and tropical tree leaves (leucaena and hibiscus) varied from 14.4 to 28.4% DM. This range was similar to that of sprouts and young leaves of evergreen species (12.2-25.5% DM). Of the evergreen species, the CP content of four species (bamboo-leaf oak, chinquapin, laurel and glossy privet) decreased by 7.5-14.0% DM with maturity. The NDFom, ADFom and ADL content of deciduous and tropical tree leaves (20.2-32.5, 16.5-27.8 and 4.3-12.0% DM, respectively) were similar to those of sprouts and young leaves of evergreen species (NDFom: 22.6-34.6% DM; ADFom: 12.3-25.7% DM; ADL: 2.4-12.2% DM), except for glossy privet. The fiber contents of bamboo-leaf oak, chinquapin and laurel increased with maturity (NDFom, ADFom and ADL increased by 23.8-29.6, 14.1-19.9, and 8.3-10.9% DM, respectively), whereas the fiber content of glossy privet decreased with maturity (NDFom, ADFom and ADL showed 10.2, 6.2 and 1.4% DM decreases, respectively).

The NFC contents of each tree species ranged from 30.8% to 57.4%, with no clear differences between tree types. However, mature leaves of deciduous trees had numerically higher NFC content (Yoshino cherry: 54.1% DM; willow: 48.0% DM). Among evergreen trees, NFC content decreased with maturity in bamboo-leaf oak, chinquapin and laurel (young leaves and sprouts: 46.0-57.4% DM; mature leaves: 30.8-31.5% DM), whereas NFC content increased with maturity in glossy privet (young leaves: 46.8% DM, mature leaves: 52.3% DM).

The Ca content of deciduous and tropical tree leaves ranged from 1.14 to 2.24% DM (Appendix T4). The Ca content of sprouts and young leaves of four evergreen tree species were relatively low (0.24-0.76% DM); however, they increased with leaf age (0.94-4.41% DM). The leaf P content was not affected by tree type, but the content decreased with leaf age in bamboo-leaf oak, chinquapin, laurel and glossy privet. The Ca/P ratio in sprouts and/or young leaves of the four evergreen tree species ranged from 0.5 to 2.3, whereas the Ca/P ratio in the remaining tree species was considerably high (3.7-33.9; Fig. 3).

Modification of diet regimen

The following findings refer to the diet without the leaves. The amount of Ca and P contained in the diet without leaves consumed by Genki were 1.5-2 times higher after dietary modification than prior to it, i.e., 350±91 mg Ca/100 g FM (range: 251-496 mg/100 g FM; n = 7) and 314±45 mg P/100 g FM (range: 262-383 mg/100 g FM; n = 7) to 534±293 mg Ca/100 g FM (range: 335-871 mg/100 g FM; n = 3) and 705±285 mg P (range: 511-1032 mg; n = 3) (Fig. 4). The Ca/P ratio in the diet without leaves was reduced from 1.11 in the previous diet (range: 0.95-1.32; n = 7) to 0.73 in the modified diet (range: 0.65-0.84; n = 3) (Fig. 4), indicating that the Ca/P ratio was consistently below 1.0 without leaves. Additionally, we increased ME supply in the diet to prevent energy deficiency in Genki; the increased quantity of soybeans and peanuts raised the ME content from 298±36 kcal/100 g FM (range: 258-345 kcal/100 g FM; n = 7) to 579±212 kcal/100 g FM (range: 413-818 kcal/100 g FM; n = 3) (Fig. 4).

Effects of dietary modification

Body weight

As shown in Figure 1, the body weight of Kinanty tended to decrease in winter, but it showed a clear trend of increasing until the female gave birth for the first time in 2012. Thereafter, the trend of decreasing weight in winter generally remained unchanged, but the weight of Kinanty remained relatively stable until giving birth for the second time in 2015; note that there was an increase in weight shortly before giving birth. After the second birth, there was still a tendency for a decreased body weight in winter compared to other seasons, until the food modification was implemented. Likewise, the body weight of Genki showed an overall trend of increasing, but temporary weight loss every winter was generally observed until 2017, when the dietary modification started. As for the other individuals, the basic trend appeared to be the same: a temporary loss of weight in the winter (Figure 1).

Plasma Ca, inorganic P and Ca/P ratio

Prior to the dietary modification, the plasma Ca and P concentrations of the two individuals were not significantly different in summer ($n = 9$ from April to October, Ca: 12.9±1.3; P: 3.7±1.7) nor in winter ($n = 11$ from November to March, Ca: 14.0±1.3; P: 3.3±2.3) (Ca: $U = 28.0$ and $p = 0.110$; P: $U = 60.5$, $p = 0.425$). Plasma Ca concentrations in Kinanty and Genki after dietary modification (Kinanty: 9.8±0.7 mg/dL, $n = 3$; Genki: 10.4±2.1 mg/dL, $n = 3$) were lower than those prior to the modification (Kinanty: 12.8±1.4 mg/dL, $n = 10$; Genki: 14.1±1.5 mg/dL, $n = 10$). The plasma P concentration in Kinanty was not different after the modification (3.7±1.9 mg/dL, $n = 3$) from the value before (3.6±2.1 mg/dL, $n = 10$). The mean P concentration in Genki decreased by almost half from before (3.3±1.9 mg/dL, $n = 10$) to after (1.9±0.7 mg/dL, $n = 3$) the modification. The overall tendency was that the plasma Ca concentration was significantly decreased by dietary modification ($U = 110.5$, $p = 0.002$), although the plasma P concentration and Ca/P ratio did not change significantly before and after dietary modifications (P: U = 74.0 and p = 0.411; Ca/P ratio: U = 64.0 and p = 0.831).

Comparison of plasma Ca and P concentrations between zoo and wild individuals

The plasma Ca and P concentrations of proboscis monkeys under natural conditions were not significantly different from those under provisioned conditions (Ca: U = 112.0, p = 0.463; P: U = 150.0, p = 0.691). The plasma Ca concentration of zoo proboscis monkeys before the dietary modification was significantly higher than those of animals in the natural (U = 709.0, p < 0.001) and provisioned conditions (U = 0.0, p < 0.001). On the other hand, the plasma Ca concentrations of zoo animals after the dietary modification were not significantly different from those of animals in the natural (U = 54.0, p = 0.037) and provisioned conditions (U = 14.0, p = 0.353), indicating that the dietary modification successfully decreased the plasma Ca concentration to a level comparable to that of free-ranging individuals (Fig. 5).

However, the plasma P concentration and Ca/P ratio of zoo proboscis monkeys before and after dietary modifications were significantly different from those measured in the natural condition (i.e., P concentration before modification: U = 709.0, p < 0.001; P concentration after modification: U = 222.0, p < 0.001; Ca/P ratio before modification: U = 29.0, p < 0.001; Ca/P ratio after modification: U = 12.0, p = 0.006) or the provisioned condition (i.e., P concentration before modification: U = 127.0, p = 0.002; P concentration after modification: U = 41.0, p = 0.005; Ca/P ratio before modification: U = 139.0, p < 0.001; Ca/P ratio after modification: U = 41.0, p = 0.005) (Fig. 5). In other words, diet modification was not sufficiently effective in increasing the plasma P concentration and decreasing the Ca/P ratio of zoo proboscis monkeys.

Discussion

In general, pathological findings in colobines have often highlighted gastrointestinal disorders associated with infections (Loomis et al. 1983; Palmieri et al. 1984; Pang et al. 1993; Overskei et al. 1994; Nishiura et al. 2019), probably due to the distinctive foregut fermentation capability of their digestive system. Hence, renal disease in colobines has not been extensively documented, and the treatment of such disease has not been well established, although it is considered one of the significant causes of death or of underlying long-term health problems in colobines (Janssen 1994; Shelmidine et al. 2013). In a recent study by Baker et al. (2022), providing the most comprehensive review of renal disease in Asian colobines, urinary retention due to urinary calculi was associated with substantial morbidity and mortality, thus emphasizing the need for further research into its etiology and preventive medicine. Therefore, our detailed report of four cases of renal disease in captive proboscis monkeys adds to a basis for future medical treatment and management of the disease.

Weight loss in relation to diet

Free-ranging proboscis monkeys inhabiting natural habitats generally prefer to feed on young leaves rather than mature leaves throughout the year (Yeager 1989; Boonratana 2003; Matsuda et al. 2009; Bernard et al. 2019); however, in zoos, especially those located in temperate regions with four seasons, nutritional status and leaf maturity in available plant species vary across the year (Nijboer and Dierenfeld 1996; Kawasaki et al. 2021; Hoshino et al. 2021). Consequently, zoo animals are generally healthy in spring and summer, when they are fed a relatively steady supply of young leaves, but in autumn and winter, when this is more difficult, the monkeys are more prone to weight loss due to the increased supply of lower energy diets, such as mature leaves of evergreen plants. In fact, the NFC content in tree leaves was relatively high in mature leaves of deciduous trees and young evergreen leaves, which could be fed in spring and summer, but relatively low in mature evergreen leaves, which were fed in autumn and winter, suggesting that seasonal changes in energy amounts may have caused weight loss.

In addition, since the air temperature in natural habitats inhabited by proboscis monkeys ranges from approximately 23 to 32 °C throughout the year (Matsuda et al. 2011), proboscis monkeys housed at YZG are likely to suffer from cold stress, as the room temperature is usually kept at 20-25 °C in winter. The lower critical temperature for proboscis monkeys is poorly known, but given that all individuals consistently lost weight in winter before the dietary modification was introduced in 2017, it is plausible that the supply of ME was merely inadequate for such indoor conditions. Thus, in addition to dietary modification, monitoring the ambient temperature may be an important factor in preventing weight loss in captive proboscis monkeys during autumn and winter in temperate regions.

Renal failure in relation to diet

Given that there was no seasonality in plasma Ca and P levels before the dietary modification, it could be assumed that the zoo proboscis monkeys had high plasma Ca with low plasma P throughout the seasons. Potential contributors to hypercalcemia and/or hypophosphatemia comprise a relative imbalance of Ca, P and vitamin D (Crissey et al. 1998; Goff 2000; Kato et al. 2004; Schubert and Deluca 2010; Cline 2012) and primary or secondary elevations in parathyroid hormone or parathyroid hormone-related protein, which can occur as part of renal disease or paraneoplastic syndromes (Goff 2000; Carroll and Schade 2003; Santos et al. 2013), respectively. However, our clinical reports did not detect potential triggers of hypercalcemia or hypophosphatemia, such as neoplasia or parathyroid abnormalities, exposure to vitamin D supplements or special exposure to UV radiation. This ruled out factors other than the diet as the cause of the high plasma Ca and low plasma P in the zoo proboscis monkeys.

On the other hand, the ratio (Ca/P) of the diet without leaves was adjusted to be lower than before the modification, but the Ca and P in the diet after the modification increased, thereby successfully reducing the plasma Ca level (although it failed to increase the P level). Thus, rather than simply adjusting the balance between the Ca and P levels in the diet, the increase in their absolute amount might be considered to be more important; this is also consistent with the fact that not only

Ca/P imbalance but also the requirements of Ca and P of diets have been identified as a factor causing renal disease in many captive foregut-fermenters, not just colobines (Emerick and Embry 1963; Rappaport and Hochman 1988; Lindemann et al. 2013; Han and Garner 2016). Note, however, that as in the case of foregut fermenting goats, hypophosphatemia associated with the appearance of kidney stones is unexplained and uncontrolled (George et al. 2007); thus, there may be cases where treatments other than simply modifying absolute amounts of Ca and P levels and their ratios and other mineral balance (e.g., vitamin D supplementation) might need to be considered.

In the present study, when the animals consumed mature leaves of Yoshino cherry, willow, leucaena and hibiscus at YZG, the Ca/P ratio (3.7-8.2) was approximately two to eight times higher than the recommended value (1-2) for primates in general (NRC 2003); it has been reported that the Ca/P ratio in the leaves preferred by free-ranging proboscis monkeys is 2 (Yeager et al., 1997). On the other hand, the Ca/P ratio in sprouts and/or young leaves of bamboo-leaf oak, chinquapin and laurel in the present study were comparable to those in the leaves eaten by free-ranging proboscis monkeys, i.e., Ca: 0.24-0.49% DM at YZG vs. 0.35% DM in the wild (Yeager et al. 1997); P: 0.29-0.51% DM at YZG vs. 0.15% in the wild (Yeager et al. 1997). Thus, considering the nutritional profile of the diets supplied to zoo proboscis monkeys, we cannot deny the possibility that renal failure occurred because the leaves, especially fed in autumn and winter, had a much higher Ca/P ratio than is appropriate. Indeed, no renal disease has been reported to date under the modified diet regimen (A.A. 2022 pers obs), supporting the possibility that inappropriate diets were the major cause of kidney failure in proboscis monkeys at YZG.

In free-ranging proboscis monkeys, leaf toughness and/or color, assessed by oral and visual sensations may be a proximate cue for the content of nutrients such as protein and fiber (Matsuda et al. 2017), which are important determinants of their dietary choice (Yeager et al. 1997; Boonratana 2003; Matsuda et al. 2013), but they are probably no cues for minerals such as Ca and P. Accordingly, dietary planning for zoo animals would require consideration not only of their preferences but also of appropriate nutritional balance, including mineral content, in browse plants.

Evaluation of dietary modification

The present study indicates that dietary modification in terms of mineral proportion and ME achieved beneficial effects on proboscis monkeys in a captive environment, but unfortunately the measurements were taken of the diet of only two animals with no control group. After dietary modification, the body weight of Kinanty and Genki recovered to a level comparable to that of free-ranging conspecifics (male: 19.7-25.2 kg; female: 9.3-13.8 kg, Bismark 2010; Matsuda et al. 2020), and both individuals were able to maintain their body weight throughout the autumn and winter of 2018 (Fig. 1), indicating that the ME supply in the improved diets was close to the *ex situ* energy requirement. Moreover, the modified diets in zoo proboscis monkeys appear to successfully reduce the plasma Ca concentration (10.1 mg/dL), which was similar to that of free-ranging conspecifics (6.6-13.3 mg/dL) (Fig. 5) and other colobine species such as guereza (*Colobus guereza*), i.e., 7.9-10.9 mg/dL (Miller and Fowler 2015). In the zoo proboscis monkeys described in the present study, no renal disease or related clinical symptoms have occurred to date after dietary modification (A.A. 2022 pers obs), suggesting that adjusting the amount of Ca and P in the diet can prevent diseases such as renal failure. On the other hand, the present study also exposed the difficulty of improving physiological status by modifying diets; the change in the amount of P in the diet of the proboscis monkeys was not clearly reflected by their plasma P (Fig. 5). This suggests that it is necessary to adjust not only the balance of several minerals but also the amount of each mineral to rectify the imbalance of minerals in plasma. Consequently, for more appropriate dietary improvement in the future, it is important to determine the energy requirements and mineral requirements of the animals and to feed them with particular attention to the ratio of mineral components.

Implication of diet regimens for proboscis monkeys in temperate regions

For captive folivorous primates, especially colobines, the recommendation often calls for more foliage in their diets (Matsuda et al. 2018), but providing young leaves of evergreen species year-round is not practical in temperate regions. On the other hand, the leaves of some deciduous and imported tropical tree species in the region were not suitable feedstuffs in terms of their Ca/P ratio (i.e., lower P contents), although captive proboscis monkeys preferred to eat those leaves. Therefore, we advise caution in designing diet regimens for colobines housed in temperate regions; feedstuffs should not be chosen simply on the basis that they are foliage or that the animals prefer to ingest them. Modifying the amount of Ca and/or P and ME using commercial foods, e.g., mineral- and/or energy-rich leafy vegetables, may be a feasible way to appropriately control energy supply and chemical/mineral balance in the whole diet to prevent weight loss, hypercalcemia, hypophosphatemia, and possible renal disease in captive colobines.

Acknowledgments

We thank all staff and veterinarians at YZG who are committed to managing and improving the health management of proboscis monkeys; we are especially grateful to Miya Ueda and Ryuta Kawasaki. We also thank the Sabah Biodiversity Centre, the Sabah Forestry Department, and the Sabah Wildlife Department for granting us permission to conduct this research in Sabah, Malaysia, and the Wildlife Rescue Unit rangers for their assistance in the field. We are grateful for the support from Dr. Danica J. Stark, Dr. Rosa Sipangkui and our research assistants in the field. We would also like to thank Marcus Clauss as well as an unknown reviewer for their very helpful comments on this manuscript. Finally, we would like to commemorate the four monkeys that died of renal disease.

References

AOAC (2005) Official methods of analysis, 18th edn. Association of Official Analytical Chemists, Arlington, VA, USA

Atwater WO (1910) Principles of nutrition values of food. United states farmer's bulletin 142

Azumano A, Ueda M, Tanaka S (2015) Report of a fatal case of a juvenile proboscis monkey (*Nasalis larvatus*). Bulletin of the zoological studies 13:30–33 (in Japanese)

Baker KC, Georoff TA, Ialeggio DM, et al (2022) Retrospective review of urolithiasis-related morbidity and mortality in Asian colobine monkeys. J. Zoo Wildl. Med. 53:1-10. https://doi.org/10.1638/2021-0023

Bernard H, Matsuda I, Hanya G, et al (2019) Feeding ecology of the proboscis monkey in Sabah, Malaysia, with special reference to plant species-poor forests. In: Primates in flooded habitats: Ecology and conservation. Cambridge University Press, Cambridge, pp 89–98

Bismark M (2010) Proboscis monkey (*Nasalis larvatus*): Bio-ecology and conservation. In: Indonesian primates. Springer, New York, pp 217–233

Boonratana R (2003) Feeding ecology of proboscis monkeys (*Nasalis larvatus*) in the lower Kinabatangan, Sabah, Malaysia. Sabah Parks Nature Journal 6:1–6

Calle P, Raphael B, Stetter M, et al (1995) Gastrointestinal linear foreign bodies in silver leaf langurs *Trachypithecus cristatus ultimus*. J. Zoo Wildl. Med. 26:87–97

Carroll MF, Schade DS (2003) A practical approach to hypercalcemia. Am Fam Physician 67:1959–1966

Clauss M, Dierenfeld E (2008) The nutrition of browsers. In: Zoo and wild animal medicine: Current therapy, 6th edn. Saunders Elsevier, St. Louis, pp 444–454

Cline J (2012) Calcium and Vitamin D Metabolism, Deficiency, and Excess. Top Companion Anim Med 27:159–164. https://doi.org/10.1053/j.tcam.2012.09.004

Collins L, Roberts M (1978) Arboreal folivores in captivity- maintenance of a delicate minority. In: The ecology of arboreal folivores. Smithsonian Institution, Washington DC, pp 5–12

Crissey S, Pribyl L, Pruett-Jones M, Meehan T (1998) Nutritional management of old world primates with special consideration for vitamin D. Int. Zoo Yearb. 36:122–130. https://doi.org/10.1111/j.1748-1090.1998.tb02894.x

Dierenfeld ES, Koontz FW, Goldstein RS (1992) Feed intake, digestion and passage of the proboscis monkey (*Nasalis larvatus*) in captivity. Primates 33:399–405. https://doi.org/10.1007/BF02381201

Edwards MS, Crissey SD, Oftedal OT (1997) Leaf-eating primates: nutrition and dietary husbandry. NAG handbook Fact Sheet 7

Emerick R, Embry L (1963) Calcium and phosphorus levels related to the development of phosphate urinary calculi in sheep. J. Anim. Sci. 22:510–513. https://doi.org/10.2527/jas1963.222510x

Ensley P, Rost T, Anderson M, et al (1982) Intestinal obstruction and perforation caused by undigested Acacia sp leaves in langur monkeys. J. Am. Vet. Med. Assoc. 181:1351–1354

Estrada A, Garber PA, Rylands AB, et al (2017) Impending extinction crisis of the world’s primates: Why primates matter. Sci. Adv. 3:e1600946. https://doi.org/10.1126/sciadv.1600946

George JW, Hird DW, George LW (2007) Serum biochemical abnormalities in goats with uroliths: 107 cases (1992–2003). J. Am. Vet. Med. 230:101–106. https://doi.org/10.2460/javma.230.1.101

Goff JP (2000) Pathophysiology of Calcium and Phosphorus Disorders. Vet. Clin. N. Am. - Food Anim. Pract. 16:319–337. https://doi.org/10.1016/S0749-0720(15)30108-0

Han S, Garner MM (2016) Soft tissue mineralization in captive 2-toed sloths. Vet Pathol 53:659–665.

https://doi.org/10.1177/0300985815598206

Hayakawa T, Nathan SKSS, Stark DJ, et al (2018) First report of foregut microbial community in proboscis monkeys: are diverse forests a reservoir for diverse microbiomes?: Foregut microbiomes in wild proboscis monkeys. Environ. Microbiol. Rep. 10:655–662. https://doi.org/10.1111/1758-2229.12677

Hill O (1964) The maintenance of langurs (Colobidae) in captivity; Experiences and some suggestions. Folia Primatol. 2:222–231. https://doi.org/10.1159/000155018

Hollihn U (1973) Remarks on the breeding and maintenance of colobus monkeys *Colobus guereza*, proboscis monkeys *Nasalis larvatus* and douc langurs *Pygathrix nemaeus* in zoos. Int Zoo Yearb 13:185–188

Hosey G, Melfi V, Pankhurst S (2013) Zoo animals: Behaviour, management and welfare. Oxford University Press, Oxford

Hoshino S, Seino S, Funahashi T, et al (2021) Apparent diet digestibility of captive colobines in relation to stomach types with special reference to fibre digestion. PLoS One 16:e0256548. https://doi.org/10.1371/journal.pone.0256548

Inoue E, Ogata M, Seino S, Matsuda I (2016) Sex identification and efficient microsatellite genotyping using fecal DNA in proboscis monkeys (*Nasalis larvatus*). Mammal Study 41:141–148. https://doi.org/10.3106/041.041.0304

Janssen D (1994) Morbidity and mortality of douc langurs (*Pygathrix nemaeus*) at the San Diego zoo. Proceedings of the American Association of Zoo Veterinarians 221–226

Kagawa Y (2005) Standard tables of food composition in Japan 5th revised and enlarged edition.

Toppan Printing, Japan (in Japanese)

Kato Y, Sato K, Sata A, et al (2004) Hypercalcemia induced by excessive intake of calcium supplement, presenting similar findings of primary hyperparathyroidism. Endocr J 51:557–562. https://doi.org/10.1507/endocrj.51.557

Kawasaki R, Kawakita M, Kurosawa K, et al (2021) Analysis of fecal nutrients and particle size in captive proboscis monkeys (*Nasalis larvatus*) fed seasonal dietary foliage at a Japanese zoo. Anim. Sci. J. 92:. https://doi.org/10.1111/asj.13612

Kleiman D, Thompson K, Baer C (2010) Wild mammals in captivity: Principles and techniques for zoo management. 2nd edn. University of Chicago Press, Chicago

Licitra G, Hernandez TM, Van Soest PJ (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347–358. https://doi.org/10.1016/0377-8401(95)00837-3

Lindemann DM, Gamble KC, Corner S (2013) Calcium carbonate obstructive urolithiasis in a red kangaroo (*Macropus rufus*). J. Zoo Wildl. Med. 44:196–199. https://doi.org/10.1638/1042-7260-44.1.196

Loomis M, Britt J, Gendron A, et al (1983) Hepatic and gastric amebiasis in black and white colobus monkeys. J. Am. Vet. Med. Assoc. 83:1188–1191

Matsuda I, Bernard H, Tuuga A, et al (2018) Fecal nutrients suggest diets of higher fiber levels in free-ranging than in captive proboscis monkeys (*Nasalis larvatus*). Front. Vet. Sci. 4:246. https://doi.org/10.3389/fvets.2017.00246

Matsuda I, Chapman CA, Clauss M (2019) Colobine forestomach anatomy and diet. J. Morphol.

280:1608–1616. https://doi.org/10.1002/jmor.21052

Matsuda I, Clauss M, Tuuga A, et al (2017) Factors affecting leaf selection by foregut-fermenting proboscis monkeys: New insight from *in vitro* digestibility and toughness of leaves. Sci. Rep. 7:42774. https://doi.org/10.1038/srep42774

Matsuda I, Grueter CC, Teichroeb JA (2022) The colobines: Natural history, behaviour and ecological diversity. Cambridge University Press, Cambridge

Matsuda I, Stark DJ, Saldivar DAR, et al (2020) Large male proboscis monkeys have larger noses but smaller canines. Commun. Biol. 3:522. https://doi.org/10.1038/s42003-020-01245-0

Matsuda I, Tuuga A, Bernard H (2011) Riverine refuging by proboscis monkeys (*Nasalis larvatus*) and sympatric primates: Implications for adaptive benefits of the riverine habitat. Mamm. Biol. 76:165–171. https://doi.org/10.1016/j.mambio.2010.03.005

Matsuda I, Tuuga A, Bernard H, et al (2013) Leaf selection by two Bornean colobine monkeys in relation to plant chemistry and abundance. Sci. Rep. 3:1873. https://doi.org/10.1038/srep01873

Matsuda I, Tuuga A, Higashi S (2009) The feeding ecology and activity budget of proboscis monkeys. Am. J. Primatol. 71:478–492. https://doi.org/10.1002/ajp.20677

Miller E, Fowler ME (2015) Fowler’s zoo and wild animal medicine, Volume 8. Saunders

Nijboer J, Clauss M (2006) The digestive physiology of colobine primates. In: Nijboer J. Fibre intake and faeces quality in leaf-eating primates. Ridder, Utrecht University Repository, pp. 9-28. https://doi.org/10.5167/UZH-3520

Nijboer J, Dierenfeld ES (1996) Comparison of diets fed to southeast Asian colobines in North

American and European zoos, with emphasis on temperate browse composition. Zoo Biol. 15:499–507

Nishiura H, Yamazaki A, Wakakuri K, et al (2019) *Yersinia* infection in two captive guereza colobus monkeys (*Colobus guereza*). J. vet. med. sci. 81:1201–1204. https://doi.org/10.1292/jvms.19-0039

NRC (2003) Nutrient requirements of nonhuman primates, Second edition. The National Academies Press, Washington DC

NRC (2007) Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. The National Academies Press, Washington DC

Ogata M, Seino S (2015) Genetic analysis of captive proboscis monkeys: Genetic variation of captive proboscis monkeys in a Japanese zoo. Zoo Biol. 34:76–79. https://doi.org/10.1002/zoo.21176

Overskei TL, Pirie Jr. G, Veazey RS, et al (1994) Entamoeba histolytica Infection in Hanuman (*Semnopithecus entellus*) and purple-faced (*Trachypithecus vetulus*) langurs. J. Zoo Wildl. Med. 25:240–247

Palmieri J, Dalgard D, Connor D (1984) Gastric amebiasis in a silvered leaf monkey. J. Am. Vet. Med. Assoc. 185:1374–1375

Pang V, Chang C, Chang W (1993) Concurent gastric and hepatic amebiasis in a dusky leaf monkey (*Presbytis Obscurus*). J. Zoo Wildl. Med. 24:204–207

Rappaport AB, Hochman H (1988) Cystic calculi as a cause of recurrent rectal prolapse in a sloth (*Cholepus* sp.). J. Zoo Wildl. Med. 19:235. https://doi.org/10.2307/20094895

Santos F, Fuente R, Mejia N, et al (2013) Hypophosphatemia and growth. Pediatr Nephrol 28:595–

603. https://doi.org/10.1007/s00467-012-2364-9

Schubert L, Deluca HF (2010) Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. Arch. Biochem. Biophys. 500:157–161. https://doi.org/10.1016/j.abb.2010.05.029

Schwitzer C, Polowinsky S, Solman C (2009) Fruits as foods – common misconceptions about frugivory. In: Zoo animal nutrition IV. Filander Verlag, Fürth, pp 131–168

Shelmidine N, McAloose D, McCann C (2013) Survival patterns and mortality in the North American population of silvered leaf monkeys (*Trachypithecus cristatus*). Zoo biol. 32:177–188. https://doi.org/10.1002/zoo.21055

Unwin S, Ancrenaz M, Bailey W (2011) Handling, anaesthesia, health evaluation and biological sampling. In: Field and laboratory methods in primatology: A practical guide, 2nd edn. Cambridge University Press, Cambridge

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Watkins BE, Ullrey DE, Whetter PA (1985) Digestibility of a high-fiber biscuit-based diet by black and white colobus (*Colobus guereza*). Am. J. Primatol. 9:137–144. https://doi.org/10.1002/ajp.1350090207

Weatherall D (2006) The use of non-human primates in research. Academy of Medical Sciences, Medical Research Council, The Royal Society and Wellcome Trust, London, UK

Yeager CP (1989) Feeding ecology of the proboscis monkey (*Nasalis larvatus*). Int. J. Primatol.

10:497–530. https://doi.org/10.1007/BF02739363

Yeager CP, Silver SC, Dierenfeld ES (1997) Mineral and phytochemical influences on foliage selection by the proboscis monkey (*Nasalis larvatus*). Am. J. Primatol. 41:117–128. https://doi.org/10.1002/(SICI)1098-2345(1997)41:2<117::AID-AJP4>3.0.CO;2-%23

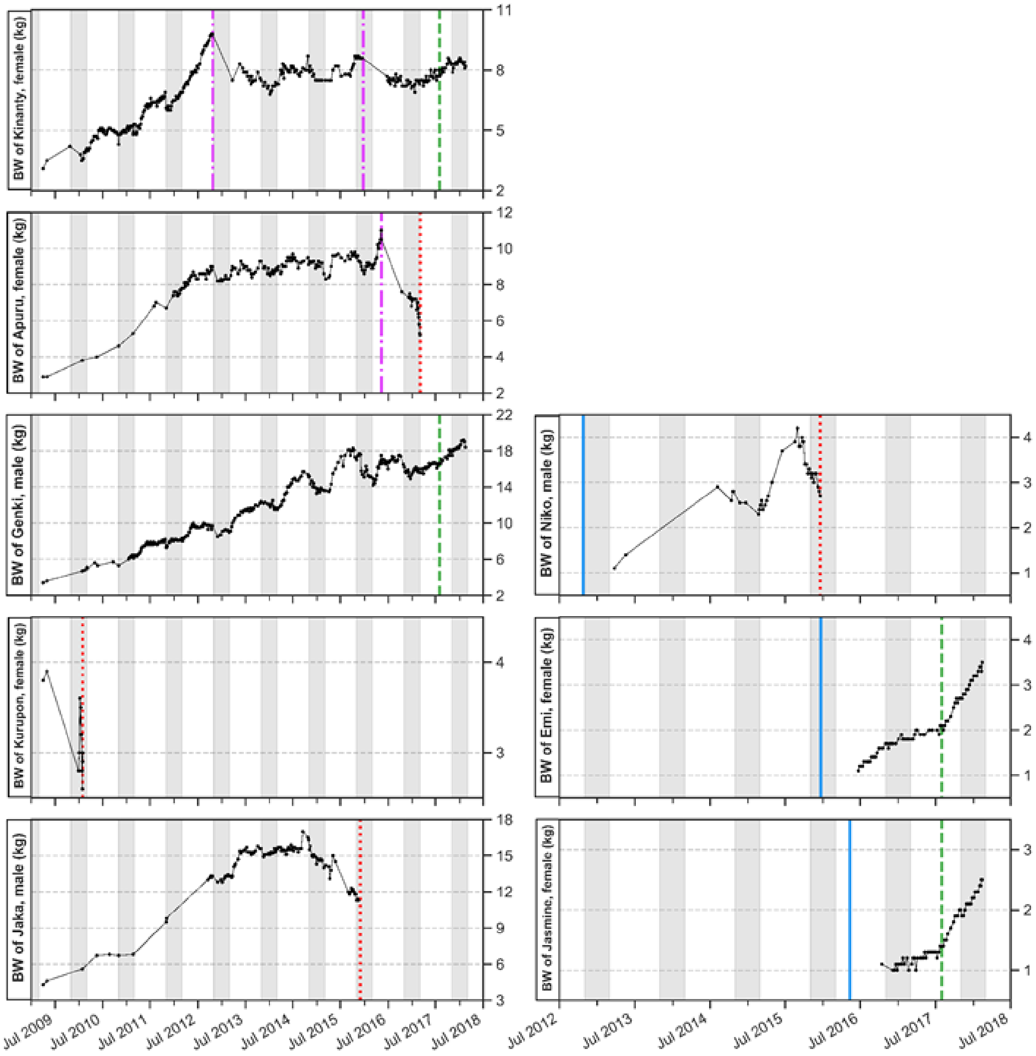


Fig 1. Body weight of eight proboscis monkeys at Yokohama Zoological Gardens (YZG), Japan. Light gray areas indicate the winter season (1 November to 1 March); a solid (blue) line indicates the day an individual was born in YZG; a dot (red) line indicates the day of death; a dash-dot (pink) line indicates the day a female gave birth; a dashed (green) line indicates the date when the modified diet was introduced.

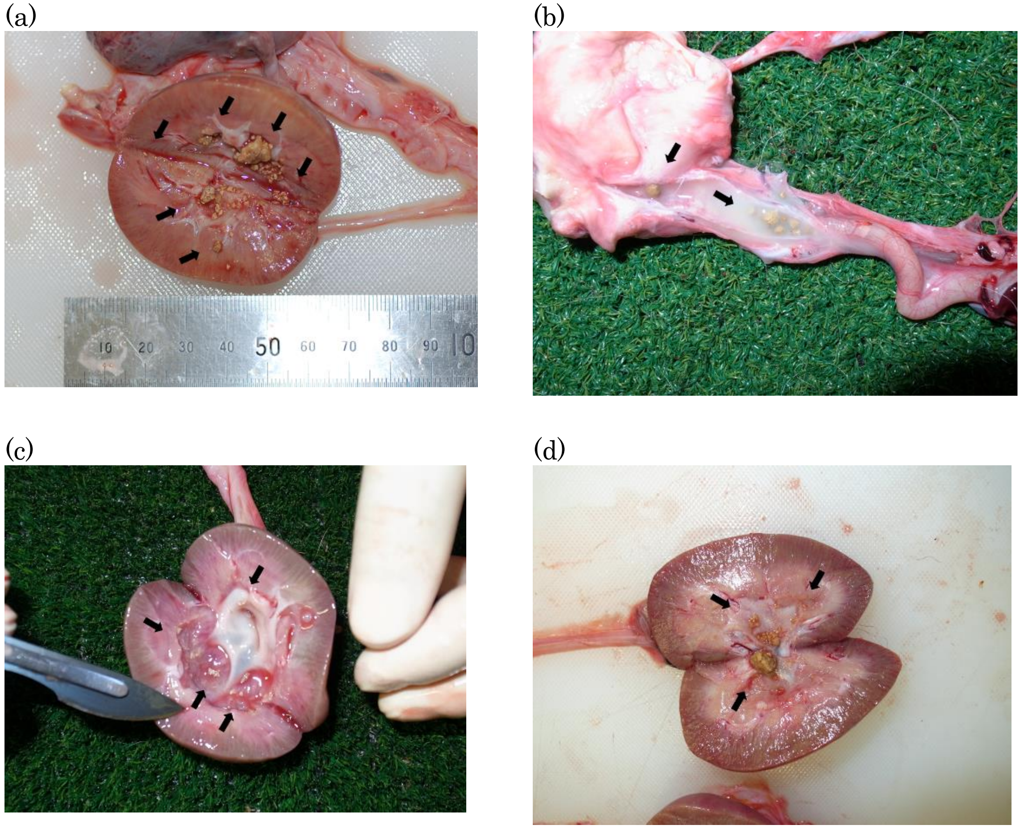


Fig 2. Kidneys and ureter with kidney stones: a) right kidney of Kurupon, b) left ureter of Jaka, c) right kidney of Niko and d) right kidney of Apuru. Arrow symbols indicate the parts in which kidney stones were found.

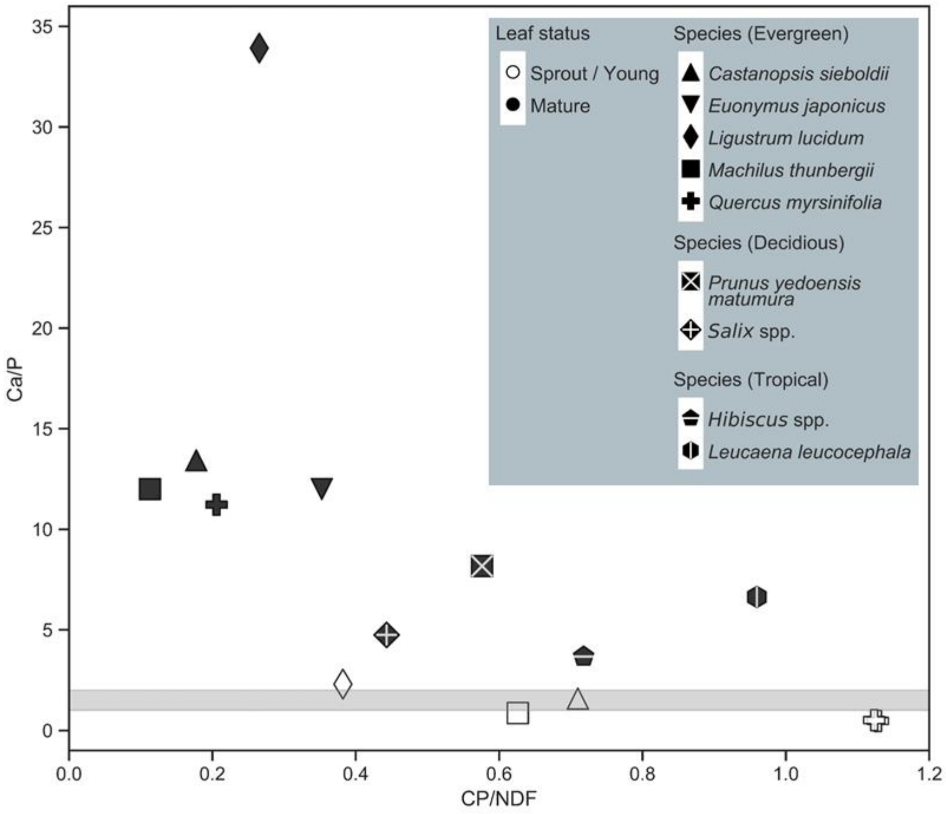


Fig 3. Scatterplot of the CP/NDF ratio and Ca/P ratio of 14 leaf samples: mature leaves of *Euonymus japonicus*, *Prunus yedoensis* Matsumura, *Salix* spp., *Hibiscus* spp. and *Leucaena leucocephala*; young and mature leaves of *Castanopsis sieboldii*, *Ligustrum lucidum* and *Machilus thunbergii*; sprouts, young leaves and mature leaves of *Quercus myrsinifolia*.

White and black symbols indicate sprouts/young leaves and mature leaves, respectively. Each symbol type represents a different browse species (five evergreen, two deciduous and two tropical species). The commonly recommended range of Ca/P ratios between 1 and 2 (NRC 2003, 2007) is represented by the gray line.

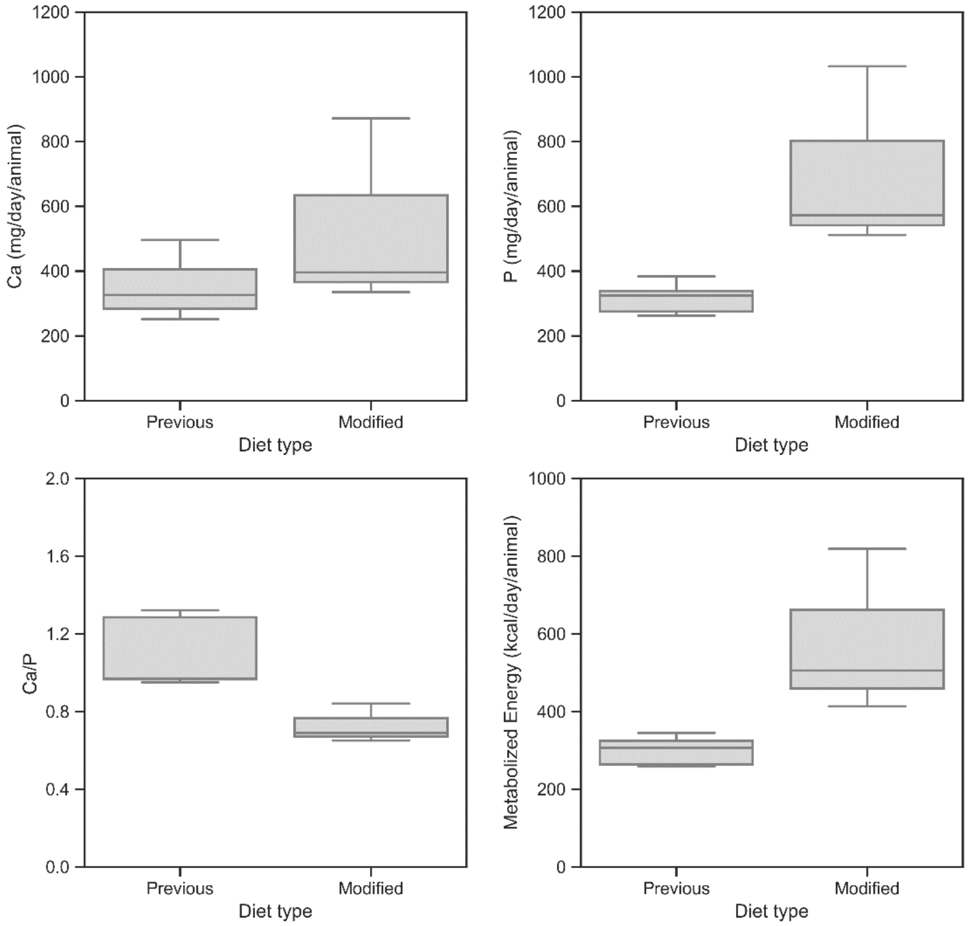


Fig 4. Ca and P content, total Ca/P ratio and metabolized energy (ME) fed to Genki on the previous (n = 7) and modified (n = 3) diets without leaves.

For each diet, the Ca, P and ME content of commercial feeds were referred to in standard tables of food composition (Kagawa, 2005).

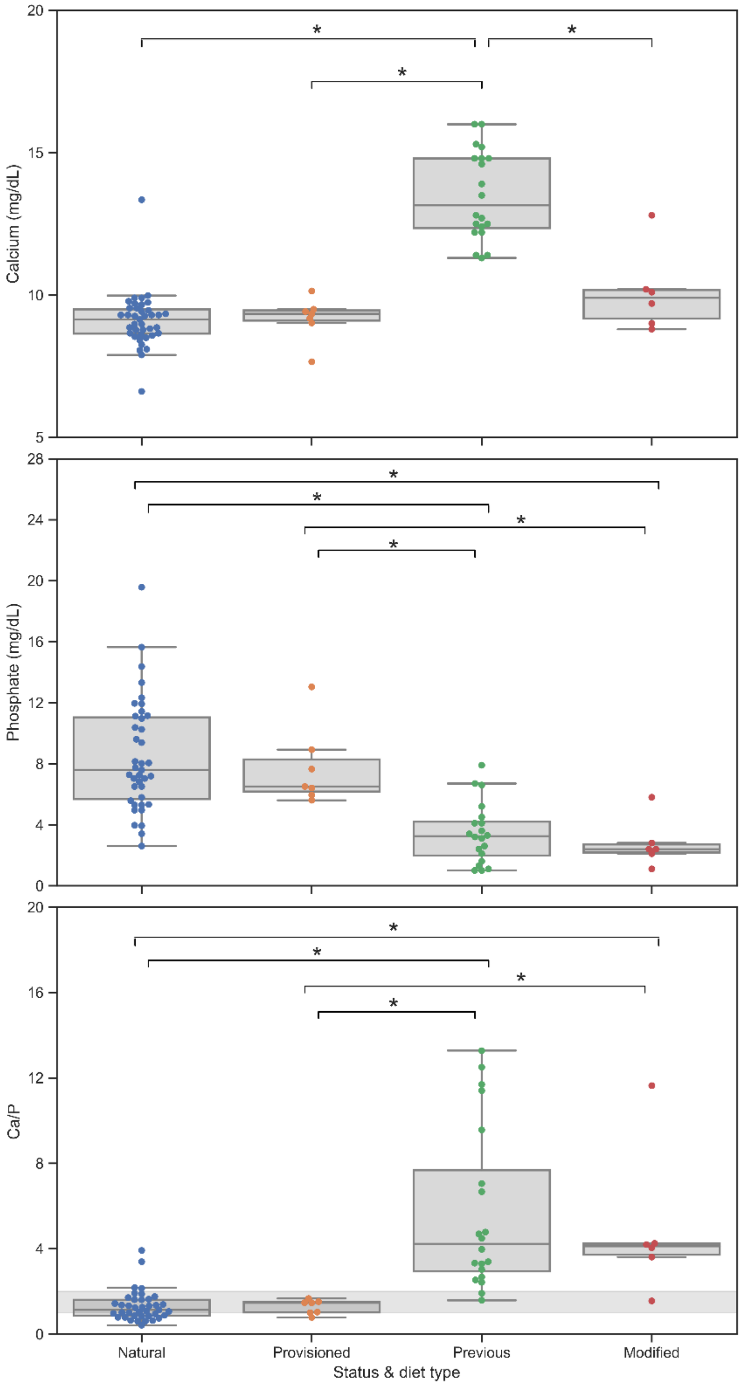
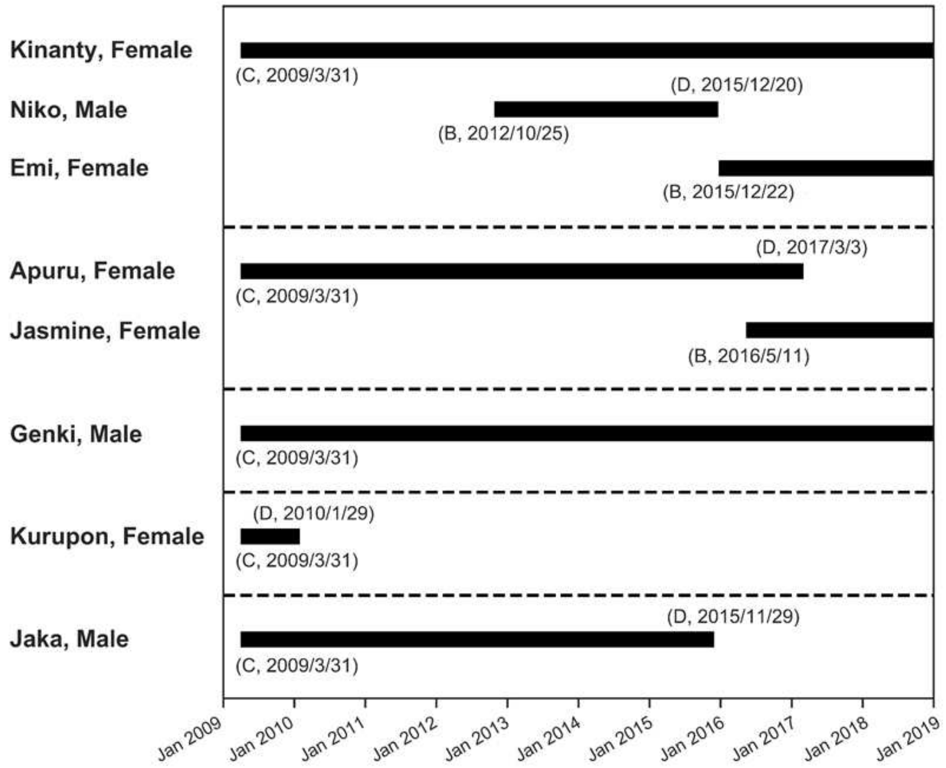


Fig 5. Boxplots illustrating variation in plasma Ca and P concentrations (mg/dL) and Ca/P ratio, with each point representing the individual plasma Ca content (upper), P content (middle) and Ca/P ratio (bottom) of proboscis monkeys, the central line represents the median, and the lower and upper

bounds of the box represent the first and third quartiles.

Natural: free-ranging individuals consuming only natural diets (n = 39); Provisioned: free-ranging individuals consuming a combination of natural and artificial foods (n = 7); Previous: two zoo individuals (Kinanty and Genki) before dietary modification (n = 20); Modified: two zoo individuals (Kinanty and Genki) after dietary modification (n = 6).

* Significant difference. In the comparisons of Ca and P levels and Ca/P ratios between “Previous” and “Modified”, significance was defined as a p value below 0.05. When these variables were compared between zoo (“Previous” or “Modified”) and free-ranging (“Natural” and “Provisioned”) proboscis monkeys, significance was defined as a p value below 0.018; this threshold was set using the Bonferroni correction (0.05/N, N = 3).



Appendix F1. Individual status of eight proboscis monkeys at YZG, Japan.

Niko and Emi are the infants of Kinanty × Jaka, respectively. Jasmine is the infant of Apuru × Genki.

B indicates the day an animal was born at YZG; C indicates the day an animal came from the Surabaya Zoo, Indonesia; and D indicates the date of death.

Appendix T1. Feedstuffs and parts of each plant species provided at YZG, with sampling dates.

Tree type	Species	Sampling parts	Sampling dates
Deciduous	Yoshino cherry (*Prunus yedoensis* Matsumura)	Mature leaf	May 7, 2017
			May 11, 2017
			May 15, 2017
	Willow (*Salix* spp.)	Mature leaf	May 3, 2017
			May 6, 2017
			May 12, 2017
Evergreen	Bamboo-leaf oak (*Quercus myrsinifolia*)	Sprout	May 5, 2017
		Young leaf	May 14, 2017
		Mature leaf	May 14, 2017
	Chinquapin (*Castanopsis sieboldii*)	Sprout	May 5, 2017
			May 12, 2017
		Mature leaf	May 5, 2017
			May 12, 2017
	Laurel (*Machilus thunbergii*)	Sprout	May 6, 2017
		Mature leaf	May 5, 2017
			May 12, 2017
	Glossy privet (*Ligustrum lucidum*)	Sprout	May 5, 2017
			May 14, 2017
		Mature leaf	May 5, 2017
			May 14, 2017
	Japanese spindle tree (*Euonymus japonicus*)	Mature leaf	Nov 14, 2017
Tropical	Leucaena (*Leucaena leucocephala*)	Mature leaf	Oct 16, 2017
			Nov 5, 2017
	Hibiscus (*Hibiscus spp.*)	Mature leaf	Oct 16, 2017
			Nov 5, 2017

Appendix T2. Ca, P and ME content in the diet of a captive proboscis monkey (Genki) at YZG before and after dietary modifications.

a) Nutrient contents of each feedstuff in each diet

	Before modification * (n = 7)							After modification * (n = 3)		
	Jun 2, 2016	Jul 25, 2016	Nov 1, 2016	Dec 1, 2016	Mar 20, 2017	May 17, 2017	Jul 4, 2017	Jul 31, 2017	15 Aug, 2017	Oct 16, 2017
Amount of each feedstuff (g FM)										
Carrot	80	100	100	100	100	100	120	120	120	120
Green bean	160	280	280	240	240	180	240	240	120	160
Cucumber	160	200	200	160	160	120	200	200	200	100
Apple	160	220	220	180	180	160	200	240	120	160
Japanese mustard spinach	0	0	100	80	80	0	0	0	0	0
Broccoli	0	0	0	0	0	0	0	100	100	140
Asparagus	0	0	0	0	0	0	0	100	100	100
Soybean	0	0	0	0	0	0	0	30	30	40
Commercial pellet (Primate L/S Banana Biscuit, Mazuri)	10	10	10	10	5	10	10	10	10	60
Peanut	10	10	10	10	5	10	10	20	20	60
Total nutrient contents										
Feed amount (g FM)	580	820	920	780	770	580	780	1060	820	940
Ca (mg/100 g FM)	251	326	496	431	379	255	312	396	335	871
P (mg/100 g FM)	263	338	383	339	287	262	324	572	511	1032
ME (kcal/100 g FM)	258	331	345	306	264	264	318	505	413	818

FM: fresh matter. ME: metabolizable energy.

* We changed the diet regimen for the proboscis monkeys ten times during the study period. Note that this record reflects the diet regimen as applied to one individual (Genki) in the present study.

Appendix T2. (Continued)

b) Nutrient content of each feedstuff

	Ca (mg/100 g FM)	P (mg/100 g FM)	ME (kcal/100 g FM)
Carrot	28	25	37
Green bean	48	41	23
Cucumber	26	36	14
Apple	3	10	54
Japanese mustard spinach	170	45	14
Broccoli	38	89	33
Asparagus	19	60	22
Soybean	70	190	180
Commercial pellet (Primate L/S Banana Biscuit, Mazuri)	1000	660	265
Peanut	50	380	562

The nutrient content of all feedstuffs except the commercial pellet were referred to in Kagawa (2005).

FM: fresh matter; ME: metabolizable energy.

Appendix T3. Summary of the clinical records of four proboscis monkeys that died at YZG, Japan.

Individual name	Clinical course	Autopsy findings
Kurupon	1. Lost appetite starting at the end of September 2019. 2. Kidney stones were observed on X-ray examination. 3. Died on January 29, 2010.	· Many kidney stones (diameter: 0.5-3.0 mm) in the right renal pelvis. · Several kidney stones (diameter: 0.5-1.0 mm) in the left renal pelvis. · Dilated left ureter. · Many stones (diameter: approximately 3.0 mm) obstructing left ureterovesical junction. · Interstitial nephritis and deposition of oxalate crystals in both kidneys. · No tumors or abnormalities in the parathyroid glands were identified.
Jaka	1. Medical checkup in June 2015 because he was lying on his belly with his hands on his back; bladder stones and dilated right ureter were observed. 2. The bladder stones were removed surgically on July 25, 2015, after which the monkey temporarily recovered. 3. Hospitalized on November 19, 2015, due to become low-spirited 4. Died on November 29, 2015.	· In the right kidney, there were no kidney stones but renal atrophy and damage to renal parenchyma were found. · The renal capsule of the left kidney was markedly inflamed and adhered to the pancreas. · There were purulent materials around and under the renal capsule of the left kidney, and the surface bled after the capsule was peeled off. · No urine in the bladder. · The left ureter opening was clogged with stones. · Approximately 50 stones (diameter: approximately 1.0-5.0 mm) were found from the bladder to the left renal pelvis. · Suppurative nephritis, suppurative pyelitis, suppurative perinephritis and renal atrophy with fibrosis. · No tumors or abnormalities in the parathyroid glands were identified.

Appendix T3. (Continued)

Niko	1. Stones were found in the right kidney in February 2015 (Azumano et al. 2015); the monkey was treated and observed. 2. Anemic condition deteriorated starting in November 2015. 3. Fell down due to hypoglycemia on December 15 and December 19, 2015. 4. Recovered temporarily after medical treatment on December 19, 2015 but died the next morning.	· The right kidney had a dilated renal pelvis and contained approximately 10 kidney stones (diameter: approximately 1.0-3.0 mm). · The right ureter was dilated, and its opening was blocked by kidney stones. · The left kidney had a dilated renal pelvis and contained a 2.0 mm kidney stone. · The left ureter was dilated, but no kidney stone was found at its opening; however, the left ureterovesical junction was congested and swollen. · Stones lodged inside each renal pelvis. · No change in the color or structure of both kidneys. · 4-5 stones (diameter: approximately 1.0-2.0 mm) in bladder, potentially due to inflow of stones through the right ureter at autopsy. · Renal interstitial fibrosis and crystal deposition in tubular epithelium (Azumano et al. 2015). · No tumors or abnormalities in the parathyroid glands were identified (Azumano et al., 2015).

Appendix T3. (Continued)

Apuru	1. Ureterotomy on October 14, 2016; laparotomy and ureteral approach on February 7, 2017. 2. Developed a pneumothorax after the operation. 3. The air in the pneumothorax was removed on February 17, 2017, and good progress was observed postoperatively. 4. Gradually weakened and lost weight; died on March 3, 2017.	· Subcapsular hemorrhage in the right kidney. · The right ureter was not dilated, but sand-like kidney stones were found in the proximal ureter. · Part of the renal capsule of the right kidney adhered to the intestinal tract and liver. Kidney stones (a 5-6 mm stone and many sand-like stones) were found in the right renal pelvis. · The left kidney did not contain kidney stones but showed irregularity in shape, and the left ureter was observed to be dilated. · The color of the cortex of left kidney was lighter than that of the right kidney. · The capsules of both kidneys were peeled off easily, and the parenchyma had become sclerotic. · Tubular crystal deposition and cast formation with mild interstitial fibrosis. · No tumors or abnormalities in the parathyroid glands were identified.

Appendix T4. Nutrient content of tree leaves fed at YZG, Japan.

Tree type	Leaf species	Sampled part	Sampled part	DM (%)	CP (% DM)	NDICP (% CP)	ADICP (% CP)	NDFom (% DM)	ADFom (% DM)	ADL (% DM)
Deciduous plant	Yoshino cherry (*Prunus yedoensis* Matsumura)	Mature leaf	Leaf	29.9	15.5	41.2	11.2	26.9	18.8	6.5
	Willow (*Salix* spp.)	Mature leaf	Leaf	31.7	14.4	31.8	26.3	32.5	27.8	12.0
Evergreen plant	Bamboo-leaf oak (*Quercus myrsinifolia*)	Sprout	Sprout	25.5	25.5	13.9	4.8	22.6	12.3	2.9
		Young leaf	Young leaf	27.4	24.6	16.5	6.6	21.9	15.6	2.4
		Mature leaf	Mature leaf	48.4	10.6	60.2	22.0	51.5	31.6	10.7
	Chinquapin (*Castanopsis sieboldii*)	Sprout	Sprout	25.1	18.1	19.4	9.2	25.5	18.7	4.7
		Mature leaf	Mature leaf	46.0	9.3	42.7	24.9	52.4	38.6	14.6
	Laurel (*Machilus thunbergii*)	Sprout	Sprout	24.1	14.9	23.2	14.2	23.8	19.4	5.9
		Mature leaf	Mature leaf	50.7	6.5	59.8	38.4	57.6	33.5	16.8
	Glossy privet (*Ligustrum lucidum*)	Sprout	Sprout	23.5	15.7	91.2	25.0	41.1	26.8	11.0
		Mature leaf	Mature leaf	32.7	8.2	86.4	32.4	30.9	20.6	9.6
	Japanese spindle tree (*Euonymus japonicus*)	Mature leaf	Leaf	33.2	12.2	39.4	11.5	34.6	25.7	12.2
Tropical plant	Leucaena (*Leucaena leucocephala*)	Mature leaf	Leaf	29.1	28.4	18.6	12.5	29.6	20.4	8.7
	Hibiscus (*Hibiscus* spp.)	Mature leaf	Leaf	20.1	14.5	12.2	5.2	20.2	16.5	4.3

DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NDFom: neutral detergent fiber without residual ash; ADFom: acid detergent fiber without residual ash; ADL: acid detergent lignin

Appendix T4. (Continued)

Tree type	Leaf species	Sampled part	NFC (% DM)	EE (% DM)	ME (kcal/g DM)	CA (% DM)	Ca (% DM)	P (% DM)	Mg (% DM)	K (% DM)
Deciduous plant	Yoshino cherry (*Prunus yedoensis* Matsumura)	Mature leaf	54.1	2.6	3.0	7.3	1.47	0.18	0.36	1.38
	Willow (*Salix* spp.)	Mature leaf	48.0	2.3	2.7	7.4	1.14	0.24	0.25	1.81
Evergreen plant	Bamboo-leaf oak (*Quercus myrsinifolia*)	Sprout	46.0	3.5	3.2	5.9	0.24	0.51	0.35	1.95
		Young leaf	49.2	2.0	3.1	6.4	0.25	0.49	0.38	2.23
		Mature leaf	31.0	4.3	2.1	9.0	1.01	0.09	0.21	0.78
	Chinquapin (*Castanopsis sieboldii*)	Sprout	52.8	1.7	3.0	5.4	0.49	0.31	0.49	1.35
		Mature leaf	30.8	5.1	2.1	6.4	0.94	0.07	0.30	0.45
	Laurel (*Machilus thunbergii*)	Sprout	57.4	2.1	3.1	5.3	0.25	0.29	0.15	1.96
		Mature leaf	31.5	2.9	1.8	5.4	0.96	0.08	0.15	0.51
	Glossy privet (*Ligustrum lucidum*)	Sprout	46.8	3.6	2.8	7.1	0.76	0.33	0.20	2.33
		Mature leaf	52.3	2.8	2.7	12.9	4.41	0.13	0.28	0.81
	Japanese spindle tree (*Euonymus japonicus*)	Mature leaf	38.4	4.8	2.5	14.8	4.32	0.36	0.23	1.08
Tropical plant	Leucaena (*Leucaena leucocephala*)	Mature leaf	34.7	4.5	2.9	8.1	1.46	0.22	0.36	0.93
	Hibiscus (*Hibiscus* spp.)	Mature leaf	48.3	4.8	2.9	14.0	2.24	0.61	0.56	1.28

NFC: non-fiber carbohydrate; EE: ether extract; ME: metabolizable energy; CA: crude ash.