

# **Short Communication**



# **Impact of Dietary Variation on Gut Microbiota Composition in** Macaca fascicularis at the Primate Research Center, IPB University

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#### ARTICLE INFO

Article history: Received June 14, 2024 Received in revised form August 27, 2024 Accepted January 13, 2025

KEYWORDS: 16S rRNA diversity, captive and semi-wild breeding, fecal samples, fruit/natural diets, long-tailed macaques, monkey chow



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### 1. Introduction

The long-tailed macaque (Macaca fascicularis; LTMs), also known as crab-eating monkeys, is a non-human primate (NHP) widely distributed across the southern part of the Southeast Asian mainland (Liedigk et al. 2015). In its natural habitat, this species spends most of its day foraging, grooming, playing, and resting at night (Hambali et al. 2012).

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#### ABSTRACT

Long-tailed macaques (Macaca fascicularis: LTMs) are widely distributed, adaptable primates commonly used as animal models in biomedical research. At primate facilities such as the Dramaga Captive Breeding Facility and the Tinjil Island Natural Habitat Breeding Facility at the Primate Research Center, IPB University, LTMs experience varying diets depending on their captive or semi-wild environments. At Dramaga Captive Breeding Facility, LTMs housed in individual and group cages are provided with different feeding regimens, whereas the semi-wild LTMs on Tinjil Island feed primarily on natural food sources with occasional, very modest provisioning of bananas. Our study's objective is to evaluate these diets' influence on the gut microbiota and overall health of LTMs. Through comparative analysis of fecal microbiomes using 16S rRNA sequencing, we identified distinct gut microbiota profiles between captive and semi-wild LTMs. Semi-wild LTMs displayed a predominance of Proteobacteria, notably Succinivibrio, associated with a high-fiber diet derived from wild plant consumption. In contrast, captive LTMs housed in individual and group cages exhibited dominance of Firmicutes and Bacteroidota, respectively. These findings suggest that diet is pivotal in shaping gut bacterial profiles, potentially impacting metabolic functions. Our study provides insight into the relationship between certain diets and gut bacterial composition in LTMs across different breeding facilities at the Primate Research Center, IPB University, thereby offering valuable implications for LTMs' welfare and management strategies.

> The captive rearing of LTMs may impact their health due to differences in habitat and diet compared to their natural environment. Captive LTMs are housed in various enclosures with restricted space for movement compared to their wild counterparts. Additionally, their diet often consists of commercial monkey chow supplemented with fruits and vegetables (Cannon et al. 2016).

> In contrast, wild LTMs primarily consume wild plant-based diets, including fruits, flowers, leaves, and occasionally small animals such as insects (Yeager 1996). These dietary disparities likely result

in differing nutritional values for captive and wild LTMs, potentially leading to obesity and diabetes in captive individuals (Bauer *et al.* 2012; Zijlmans *et al.* 2022). Moreover, captive LTMs may experience gastrointestinal issues, such as diarrhea, often associated with enteric dysbiosis or gut microbiome imbalance (Koo *et al.* 2019a; Tian *et al.* 2022).

The health of both wild and captive LTMs is closely linked to the bacterial communities within their intestines, thereby playing essential roles in host metabolism, immune system maintenance, and hormone activity (Chen et al. 2017; Martin et al. 2019). Various factors, including habitat, host genetics, and diet, influence the composition of intestinal microbiomes (Gogarten et al. 2018). Diet significantly impacts gut microbiota diversity, as shown by a study demonstrating rapid changes in gut microbiota diversity during the transfer of LTMs from the wild to captivity (Sawaswong et al. 2023). Different feeding regimes between captive and wild environments contribute to alterations in LTMs' gut microbiota composition (Boonkusol et al. 2020; Wills et al. 2022). Therefore, careful feeding management is essential for maintaining intestinal bacterial balance and the health of LTMs in captivity and the wild.

LTMs are commonly used as animal models in research and are often either bred in captivity or maintained in natural habitat breeding facilities, such as Tinjil Island, Banten Province, Indonesia. This facility is managed by the Primate Research Center, IPB University, which also encompasses the Dramaga Captive Breeding Facility. While the LTMs in semiwild conditions on Tinjil Island typically forage for natural food sources like fruits, insects, and crabs, caretaker staff also provide occasional, modest provisioning with food such as bananas or corn to the groups near the basecamp. This provisioned food is one reason the term "semi-wild" is used for the LTMs on Tinjil Island. In contrast, the LTMs in Dramaga Captive Breeding Facility are housed in specific cages and primarily fed commercial monkey chow biscuits supplemented with cultivated plant-based foods.

Previous studies have explored differences in gut microbiota composition between wild and captive LTMs (Sawaswong *et al.* 2021; Sawaswong *et al.* 2023). However, research investigating the correlation between specific diets and gut bacterial composition, particularly between breeding facilities and natural habitats in Indonesia, is limited. Only preliminary morphological-biochemical test identification has been done for LTMs' gut bacterial diversity on Tinjil Island (Tambunan *et al.* in review). Therefore, this study aimed to explore the relationship between different diets and gut bacterial profiles of LTMs in both the Dramaga Captive Breeding Facility and Tinjil Island using a molecular approach. We expect our findings to serve as an initial exploration of LTM gut bacterial profiles, offering valuable insights into primate welfare, especially regarding feeding management at the Primate Research Center, IPB University.

## 2. Materials and Methods

### 2.1. Animal and Study Sites

Three adult male long-tailed macaques (LTMs), including two captive aged 6-10 years old and one semi-wild individual, were selected for this study. The semi-wild LTMs resided at the Natural Breeding Facility of the Primate Research Centre on Tinjil Island in the Pandeglang Regency of Banten, in the southern part of Java Island (Supplementary Material, Figure S1). Here, some groups are inside the forest, while others are inhabiting the location near the caretaker staff's base camp. The captive LTMs were housed at the Dramaga Captive Breeding Facility of the Primate Research Centre, situated on the campus of IPB University in Dramaga. These captive LTMs were kept in both individual (and group cages. The individual cage was an indoor enclosure with dimensions of approximately 68 x 61 x 87 cm (length x width x height).

In contrast, the group cage used in this study was a semi-open design measuring around 20x20 m (Supplementary Material, Figure S2). This semi-open cage featured an open top covered with iron netting, allowing exposure to natural elements such as rain and sunlight. The enclosure's floor was lined with stones, facilitating the growth of small grasses. Additionally, the cage included an iron framework for the LTMs.

### 2.2. Feeding Treatment

We established feeding schedules for the LTMs housed in Individual Cage (IC\_Dramaga) and Group Cage (GC\_Dramaga), providing food twice daily, between 9-10 AM and 3-4 PM, for six months. The LTMs in IC\_Dramaga received a diet consisting solely of monkey chow, following the guidelines established by the Primate Research Centre at IPB University, with each individual receiving 200 grams per day. In contrast, the LTMs in GC\_Dramaga, comprising two adult males and 38 females, were fed a combination of monkey chow

and agricultural products, including guava, banana, and sweet potato. Each day, the LTMs would have monkey chow in the morning and agricultural products in the afternoon, and vice versa. During our fieldwork on Tinjil Island, no food was provided to the LTMs. We meticulously observed and documented the LTMs' diet in their natural habitat (SW\_Tijil). This study was conducted with the approval of the Animal Care and Use Committee (ACUC) at IPB University under protocol number IPB PRC-19-A012.

## 2.3. Fecal Sample Collection

Fresh fecal samples were collected from three macaques: one housed at IC Dramaga (in February 2023), one at GC Dramaga (in April 2023), and one from SW Tinjil, Banten (in May 2023). We used sterile falcon tubes, sterile ice cream sticks for sample collection, and 96% alcohol as a preservative solution. The inner part of the fresh fecal sample was carefully extracted to prevent contamination with soil or cage ground microbiome. Upon collection, the fecal samples from Dramaga Captive Breeding Facility were promptly transferred into ice boxes and transported directly to the laboratory, where they were stored in a refrigerator at approximately -20°C, following established protocols (Frankel et al. 2019). Similarly, the fecal samples from Tinjil Island were temporarily stored in ice boxes before transportation to the laboratory.

# 2.4. DNA Extraction and The Partial Length 16S rRNA Sequencing

DNA extraction was performed using the QiAamp DNA Mini Stool Kit (Qiagen, Germany). We assessed the concentration of DNA using the A260/280 and A260/A230 ratios measured by the NanoPhotometer NP80 (Implen, Germany). We determined the exact DNA concentration using the Qubit<sup>TM</sup> 4 Fluorometer (Invitrogen, USA), all following the manufacturer's instructions. The quality of DNA was evaluated using 1% agarose gel electrophoresis. We utilized the primers F1 (5'-CCTACGGGNGGCWGCAG-3') and R2 (5'-GACTACHVGGGTATCTAATCC-3') as developed by Klindworth et al. (2013) for amplification of the V3-V4 region, which corresponded to positions 341 to 805 in the Escherichia coli 16S rRNA gene. The expected amplicon size for this region is approximately 460-500 base pairs (bp). The 16S rRNA gene amplicon sequencing was performed using the Illumina Miseq System, and sequencing services were conducted by

a commercial sequencing company for subsequent bacterial identification.

## 2.5. Data Analysis

The Illumina Miseq Sequencing generated demultiplexed raw data, which we analyzed using the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline (Callahan et al. 2016). Cutadapt removed adapter and PCR primer sequences from the paired-end reads. Subsequently, DADA2 was utilized to correct sequencing errors, eliminate low-quality sequences, and detect chimera errors. The resulting Amplicon Sequence Variant (ASV) data were then subjected to taxonomic classification against the Silva Database (silva nr99 v138.1). R studio packages, including dada2, ggplot2, ggpicrust2, MicEco, microbiomeMarker, microbiome utilities, Microbiota Process, phyloseq, and vegan, along with Krona Tools, and PICRUSt2 were used for downstream analysis and visualizations, including alpha and beta diversity analysis.

## 3. Results

# **3.1. Food Type Per Location and Fecal Consistency Observation**

The LTMs at the IC\_Dramaga were fed only money chow, while those at GC\_Dramaga received a daily diet of monkey chow and agricultural products, such as bananas, guavas, and sweet potatoes. In contrast, the semi-wild LTMs in SW\_Tinjil were observed feeding on various wild plants, with different parts consumed (as illustrated in Table 1; Supplementary Material, Figure S4). During the sampling period, we found that the fecal samples at all locations had a normal texture or were not in liquid form (see Supplementary Material, Figure S3).

# **3.2.** Alpha and Beta Diversity of Gut Bacteria in Long-Tailed Macaques

The Shannon index revealed IC\_Dramaga (5.076) as the most diverse sample, followed by GC\_Dramaga (4.539) and SW\_Tinjil (3.376) (Figure 1A). Similarly, the Simpson index values indicated high diversity between each sample pair. Complement values of the Simpson index (1-D) demonstrated that IC\_Dramaga (0.9894) had the highest diversity index, followed by GC\_Dramaga (0.9628) and SW\_Tinjil (0.9082). These values indicate that the probability that two bacteria randomly selected from IC\_Dramaga will belong to different species is approximately 98.94%, 96.28%

for GC\_Dramaga, and 90.82% for SW\_Tinjil. Only 16 amplicon sequencing variants (ASVs) were shared across all samples, suggesting they may represent the core bacterial population in the LTMs gut environment. Furthermore, 66 ASVs were shared between IC\_ Dramaga and GC\_Dramaga, significantly higher than between Dramaga and Tinjil samples (Figure 1B). The PCoA plot based on Bray-Curtis dissimilarity showed that the gut microbiota component of LTMs in their natural habitat (Tinjil Island) differed from those raised in captivity (Dramaga Captive Breeding Facility) (Figure 1C). Moreover, even the IC\_Dramaga and GC\_Dramaga samples (representing similar captive environments) had different gut microbiota components.

# **3.3. Dominant Taxa of Fecal Bacteria in The Dramaga and Tinjil LTMs**

We observed distinct proportions of dominant bacterial phyla in each sample, with IC Dramaga comprising 52% Firmicutes, GC Dramaga containing 48% Bacteroidota, and SW Tinjil exhibiting 49% Proteobacteria (Figure 2; Supplementary Material, Figure S5). At the family level, we identified 50 bacterial families in each sample, each with varying abundance values (Supplementary Material, Figure S6). Prevotellaceae was the most abundant bacterial family in GC Dramaga, constituting 42% of the total, whereas Oscillospiraceae accounted for 23% in IC Dramaga, and Succinivibrionaceae comprised 49% in SW\_Tinjil. The most notable difference among the samples was the high abundance of Oscillospiraceae in IC Dramaga and the low Succinivibrionaceae. abundance of SW Tinjil exhibited the lowest abundance of Ruminococcaceae

Table 1. Natural items are eaten by semi-wild LTMs in Tinjil Island

and Prevotellaceae compared to the other samples. Additionally, at the genus level, *Succinivibrio* was the most abundant bacterial genus in SW\_Tinjil (49%); the UCG-002 group accounted for 14% in IC\_ Dramaga, and Prevotella\_9 constituted 29% in GC\_ Dramaga (Figure 2; Supplementary Material, Figure S7). Notably, *Succinivibrio* exhibited significantly higher abundance in SW\_Tinjil compared to the other samples, while Prevotella\_9 was most prevalent in GC\_Dramaga. Moreover, UCG\_002 or unidentified bacteria were found in exceptionally high abundance in IC\_Dramaga compared to the different samples.

## 4. Discussion

Our study revealed distinct fecal bacterial compositions in LTMs that may be due to different diets. While wild LTMs have access to a diverse array of natural food sources, including various fruits, insects, crabs, young leaves, and flowers, captive LTMs predominantly rely on commercial monkey biscuits supplemented occasionally with cultivated fruits or vegetables (Kyes 1993; Sha & Hanya 2013). Although both dietary regimes aim to meet the nutritional requirements of LTMs, variations in macronutrient content likely influence the prevalence of specific bacterial taxa associated with each diet.

When comparing potential food sources across different habitats, Tinjil Island harbors approximately 61 plant species, with 23 serving as possible food sources for semi-wild LTMs (Perwitasari-Farajallah *et al.* 2023). Semi-wild LTMs predominantly consume plants from families such as Moraceae, Malvaceae, Myrtaceae, Lecythidaceae, and Fabaceae (Santoso

Family	Species	Local name	Conservation status	Eaten parts
Asparagaceae	Dracaena elliptica	Hanjuang	Least concern	Leave
Combretaceae	Terminalia catappa	Ketapang	Least concern	Leave
Fabaceae	Intsia bijuga	Merbau	Near threatened	Bark
Gnetaceae	Gnetum gnemon	Melinjo	Least concern	Leave
Malvaceae	Thespesia populnea	Waru	Least concern	Leave
Meliaceae	Dysoxylum amooroides	Ki Langir	Data deficient	Fruit
Moraceae	Ficus hispida	Bisoro	Least concern	Fruit, Leave
Moraceae	Ficus glomerata	Ki Ara	Data deficient	Fruit, Leave
Moraceae	Ficus septica	Ki Ciat	Least concern	Fruit, Leave
Moraceae	Ficus ampelas	Ki Hampelas	Least concern	Fruit, Leave
Moraceae	Ficus variegata	Kopeng	Least concern	Fruit, Leave
Myrtaceae	Eugenia cymosa	Jambu Kopo	Data deficient	Fruit
Myrtaceae	Eugenia sp.	Jambu Lalai	Least concern	Leave
Sapotaceae	Manikara kauki	Sawo kecik	Data deficient	Fruit
Sterculiaceae	Sterculia faetida	Kepuh	Vulnerable	Fruit



Figure 1. Bacterial diversity of captive (IC\_Dramaga and GC\_Dramaga) and semi-wild (SW\_Tinjil) Long-tailed macaques. (A) Alpha diversity was determined by two indices: Shannon's diversity index, Simpson's diversity index (Complement and Inversion of Simpson), (B) The Venn diagram showed the numbers of shared taxa (ASVs) among the samples, (C) Beta diversity analysis presented by Principal coordinate analysis (PCoA) using Bray-Curtis distance



Figure 2. Krona visualization of bacterial types and abundance in the gut environment. (A) Semi-wild long-tailed macaque in Tinjil Island (SW\_Tinjil), (B) Captive long-tailed macaque in the group cage (GC\_Dramaga), and (C) Captive long-tailed macaque in the individual cage (IC\_Dramaga).

1996). Particularly favored are Ficus spp. from the Moraceae family, along with Antidesma montanum, wallichii, Melanoorhoea Barringtonia asiatica, Hibiscus tiliaceus, Eugenia cymosa, and Terminalia catappa. Notably, LTMs consume not only the fruit but also the leaves and flowers of these plants, indicating access to ample food sources on Tinjil Island and less dependency on specific fruit seasons. Natural plantbased foods are rich in dietary fiber (e.g., cellulose and hemicellulose) compared to captive diets, primarily due to the higher fiber content (around 9-18%) in wild plants (Santoso 1996). In contrast, monkey chow contains only about 3% fiber (Astuti et al. 2009). Although additional items such as cultivated fruits supplement the diets, they do not significantly increase fiber content. For instance, cultivated fruits like bananas and guava contain only 1-3% fiber (Plantain & Afolayan 2019; Hussain et al. 2021). In addition to differences in fiber content, wild food generally has lower sugar and

fat content than a captive diet, which tends to be higher in sugar (Milton 1999; Schwitzer *et al.* 2008).

Nutritional content significantly influences LTM health and is associated with intestinal microbes that aid nutrient digestion (Rinninella *et al.* 2019). Generally, wild animals have higher gut microbial diversity than captive animals due to their more complex dietary composition (McKenzie *et al.* 2017; Lugano *et al.* 2018). However, our results did not find the highest bacterial diversity in wild LTMs. This result may be because the captive LTMs (IC\_Dramaga and GC\_Dramaga) were consistently provided with a highenergy diet, such as monkey chow and agricultural items. This energy-rich diet can promote the growth of bacteria from the Firmicutes and Bacteroidetes phyla.

Meanwhile, the wild LTMs on Tinjil Island sampled in this study may not always have access to high-energy foods such as fruits due to varying fruiting seasons in the forest. This result could have influenced the limited variety of bacteria. Nevertheless, all three LTM samples exhibited high bacterial diversity, as indicated by a Shannon-Wiener H-Index value greater than 3 (Yin et al. 2019). While high bacterial diversity is generally a positive indicator of gut health, it is not the sole determinant. The overall composition, functionality, stability, and the presence or absence of pathogenic bacteria are critical factors in defining a healthy gut microbiota (McBurney et al. 2019). According to our findings, the diet of monkey chow and cultivated fruit enriched the abundance of Bacteroidota, particularly Prevotella 9, in captive LTMs. Prevotella 9 is recognized for its capability to degrade xylan polymers using endo-1,4-β-xylanase (Linares-Pastén et al. 2021). This outcome suggests that the diets provided to captive LTMs may contain higher sugar content than natural diets. Moreover, the high sugar content in monkey chow and the consumption of bananas and sweet potatoes contribute to this enrichment (Plantain & Afolayan 2019; Rodrigues et al. 2016).

A diet of monkey chow tends to elevate the abundance of the Firmicutes bacterial phylum, particularly Oscillospiraceae UCG 002 and Treponema, in IC Dramaga. These bacteria are recognized as butyrate producers and are pivotal in maintaining gut barrier integrity and anti-inflammatory activity (Donohoe et al. 2011; Knudsen et al. 2018). These bacteria aid in preventing mucosal inflammation and diarrhea in captive LTMs, which are often prevalent in captivity due to bacterial imbalances (Koo et al. 2019b). However, no instances of diarrhea were observed in the captive or semi-wild LTMs sampled in our study. Although assessing fecal consistency alone cannot definitively confirm LTM intestinal health, it can be an initial step in diagnosing diarrhea (Juckett and Trivedi 2011).

Based on our findings, both LTM populations from group and individual cages exhibited similar gut bacterial profiles, primarily dominated by Bacteroidota and Firmicutes. Captivity has been shown to impact the gut bacterial composition of non-human primates (NHPs), often resulting in a significant shift towards Bacteroidota, Firmicutes and with Prevotella predominance observed in captive NHP species (Clayton et al. 2016). However, a notable disparity was observed in the semi-wild LTMs from Tinjil Island, which displayed a higher abundance of bacteria from the Proteobacteria phylum, particularly the genus Succinivibrio. Succinivibrio is recognized for its ability to metabolize plant polysaccharides, such as starch, hemicellulose, and xylan, derived from high-fiber diets like wild fruits and leaves (De Filippo *et al.* 2017). It possesses amylase enzymes capable of breaking down indigestible starch or fiber into digestible molecules, such as propionate. Butyrate is generally more advantageous for promoting intestinal health than propionate. It serves as the primary energy source for colonocytes and is crucial in maintaining gut barrier integrity, reducing inflammation, and lowering the risk of colorectal cancer (Hosseini *et al.* 2011).

Additionally, butvrate exhibits strong antiinflammatory properties, enhancing mucin production and tight junction proteins that fortify the gut barrier. In contrast, though beneficial in regulating lipid metabolism and potentially lowering cholesterol levels, propionate has a less pronounced impact on gut health than butyrate. Nevertheless, it is important to recognize that all short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate, contribute positively to intestinal health by supporting various metabolic and immune functions (Louis and Flint 2016). This finding suggests that the LTMs from Tinjil Island may retain their indigenous gut bacterial profile, which aids in the digestion of plant-based foods.

As understood, the intestinal microbiota constitutes an integral component of our system, and their dynamic community structure can change due to various stimuli, including diets, environments, stress, genetics, and others (Ying et al. 2022). Alterations in bacterial abundance within the phylum Firmicutes and Bacteroidota significantly impact the health of NHPs in captivity. A higher abundance of Firmicutes bacteria compared to Bacteroidota is often associated with obesity in captive NPHs (Newman et al. 2021), mirroring observations in humans (Kasai et al. 2015) because Firmicutes produce more fatty acids (SCFAs), particularly butyrate, followed by propionate and acetate, resulting in increased calorie absorption compared to Bacteroidota and consequently contributing to more weight gain (Turnbaugh & Gordon 2009; Brahe et al. 2013). Although LTMs housed in individual cages tended to exhibit a higher Firmicutes/ Bacteroidota ratio, these LTMs did not display signs of obesity. However, there remains a possibility that captive LTMs in individual cages could become obese due to a lack of movement in the confined space. Meanwhile, captive LTMs in group cages also were observed not to be obese. In addition to being supported by a higher dietary fiber intake, the LTMs in group cages also enjoy more freedom of movement within the larger enclosure.

Supplementing captive LTMs' with a high-fiber diet contributes to their digestive health. Specific nutrients such as dietary fiber, amino acids, or even polyphenol compounds play significant roles in maintaining the balance of gut microbiota. These nutritional sources can help decrease the abundance of Firmicutes while increasing the abundance of Bacteroidota (Henning *et al.* 2019). Dietary fiber undergoes conversion to SCFA, which influences the gut hormone peptide YY (PYY) activity in the intestine. Consequently, this hormone regulates food intake and energy balance processing (Samuel *et al.* 2008).

Our study provides preliminary insights into gut bacteria in semi-wild LTM on Tinjil Island and captive LTMs in Dramaga. Intestinal health, particularly diet treatments, requires attention, as captive LTMs are more vulnerable to diarrhea or obesity. Nutrients and bioactive compounds influence gut bacterial profiles, highlighting the importance of diet in LTM health. One of the primary limitations of this study is the small sample size, consisting of only three adult LTMs. This limited sample may not be representative of the broader LTM population. Future research should aim to include a more extensive and diverse sample to confirm the influence of diet and environment on intestinal health and its correlation with gut bacteria at the Primate Research Center breeding locations.

#### Acknowledgements

The authors thank the Directorate General of Higher Education, Research, and Technology of The Ministry of Education, Culture, Research, and Technology for providing financial support for this study through the 2024 Research Grant Program (Contract Number: 22295/ITE.D10/PT.01.03/B/2024). We also thank the Primate Research Center, IPB University; Laboratory of Biosystematics and Animal Ecology of Biology Department; and Advanced Research Laboratory, IPB University, for permitting this study. RCK's effort was partly supported by the Office of Research Infrastructure Programs (ORIP) of the National Institutes of Health through grant number P510D010425 to the Washington National Primate Research Center, USA.

#### References

- Astuti, D.A., Suparto, I.H. Sajuthi, D., Budiarsa, I.N., 2009. Nutrient intake and digestibility of cynomolgus monkey (*Macaca fascicularis*) fed with high soluble carbohydrate diet: a preliminary study. *HAYATI. J. Biosci.* 16, 147-150. https://doi.org/10.4308/HJB.16.4.147
- Bauer, S.A., Pearl, D.L., Leslie, K.E., Fournier, J., Turner, P. V., 2012. Original Article Causes of obesity in captive cynomolgus macaques : influence of body condition, social and management factors on behaviour around feeding. *Lab. Anim.* 46, 193-199. https://doi.org/10.1258/la.2012.011120
- Boonkusol, D., Thongyuan, S., Jangsuwan, N., Sanyathitiseree, P., 2020. Antimicrobial resistance profiles in bacterial species isolated from fecal samples of free-ranging long-tailed macaques (*Macaca fascicularis*) living in Lopburi Old Town, Thailand. *Vet. World.* 13, 1397-1403. https://doi. org/10.14202/vetworld.2020.1397-1403
- Brahe, L.K., Astrup, A., Larsen, L.H., 2013. Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases ?-a review. *Obesity rev.* 7, 1-10. https:// doi.org/10.1111/obr.12068
- Cannon, T.H., Heistermann, M., Hankison, S.J., Kimberley, J., Mclennan, M.R., 2016. Tailored enrichment strategies and stereotypic behavior in captive individually housed macaques (*Macaca* spp.). J. Appl. Anim. Welf. Sci. 19, 171-182. https://doi.org/10.1080/10888705.2015.1126786
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods.* 13, 581-583. https://doi.org/10.1038/nmeth.3869
- Chen, T., Long, W., Zhang, C., Liu, S., Zhao, L., Hamaker, B.R., 2017. Fiber-utilizing capacity varies in Prevotella-versus Bacteroides-dominated gut microbiota. *Sci. Rep.* 7, 1-7. https://doi.org/10.1038/s41598-017-02995-4
- Clayton, J.B., Vangay, P., Huang, H., Ward, T., Hillmann, B.M., Al-ghalith, G.A., Travis, D.A., Long, H.T., Tuan, B.V., Minh, V.V., Cabana, F., Nadler, T., Toddes, B., Murphy, T., Glander, K.E., Johnson, T.J., Knights, D., 2016. Captivity humanizes the primate microbiome. *PNAS*. 113, 10376-10381. https://doi.org/10.1073/pnas.1521835113
- Donohoe, D.R., Garge, N., Zhang, X., Sun, W., Connell, T.M. O., Bunger, M.K., Bultman, S.J., 2011. Article the microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab.* 13, 517-526. https://doi. org/10.1016/j.cmet.2011.02.018
- De Filippo, C., Di Paola, M., Ramazzotti, M., Albanese, D., Pieraccini, G., Banci, E., Miglietta, F., Cavalieri, D., Lionetti, P., 2017. Diet, environments, and gut microbiota. A preliminary investigation in children living in rural and urban Burkina Faso and Italy. *Front. Microbiol.* 8, 1-14. https://doi.org/10.3389/fmicb.2017.01979

- Frankel, J.S., Mallott, E.K., Hopper, L.M., Ross, S.R., Amato, K.R., 2019. The effect of captivity on the primate gut microbiome varies with host dietary niche. *Am. J. Primatol.* 81, 1-9. https://doi.org/10.1002/ajp.23061
- Gogarten, J.F., Davies, T.J., Benjamino, J., Gogarten, J.P., Graf, J., Mielke, A., Mundry, R., Nelson, M.C., Wittig, R.M., Leendertz, F.H., Calvignac-Spencer, S., 2018. Factors influencing bacterial microbiome composition in a wild non-human primate community in Taï National Park, Côte d'Ivoire. *ISME J.* 12, 2559-2574. https://doi.org/10.1038/ s41396-018-0166-1
- Hambali, K., Ismail, A., Md-zain, B.M., 2012. Daily activity budget of long-tailed macaques (*Macaca fascicularis*) in Kuala Selangor Nature Park. *IJBAS- IJENS*. 12, 47–52.
- Henning, S.M., Yang, J., Woo, S.L., Lee, R., Huang, J., Rasmusen,
  A., Carpenter, C.I., Thames, G., Gilbuena, I., Tseng, C.,
  Heber, D., Li, Z., 2019. Has avocado inclusion in a weightloss diet supported weight loss and altered gut microbiota:
  A 12-week randomized, parallel-controlled trial. *Curr Dev Nutr.* 3, 1-9. https://doi.org/10.1093/cdn/nzz068
- Hosseini, E., Grootaert, C., Verstraete, W., Van de Wiele, T., 2011. Propionate as a health-promoting microbial metabolite in the human gut- a review. *Nutr. Reviews*. 69, 245-258. https:// doi.org/10.1111/j.1753-4887.2011.00388.x
- Hussain, S.Z., Naseer, B., Qadri, T., Fatima, T., Bhat, T.A., 2021. Fruits Grown in Highland Regions of the Himalayas. Springer.
- Juckett, G., Trivedi, R., 2011. Evaluation of chronic diarrhea-a handout. *Am Fam Physician*. 84, 1120-1126.
- Kasai, C., Sugimoto, K., Moritani, I., Tanaka, J., Oya, Y., Inoue, H., Tameda, M., Shiraki, K., Ito, M., Takei, Y., Takase, K., 2015. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol.* 15, 1-10. https://doi.org/10.1186/ s12876-015-0330-2
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and nextgeneration sequencing-based diversity studies. *Nucleic Acids Res.* 41, 1-11. https://doi.org/10.1093/nar/gks808
- Knudsen, K.E.B., Laerke, H.N., Hedeman, M.S., Nielsen, T.S., Ingerslev, A.K., Nielsen, D.S.G., Theil, P.K., Purup, S., Hald, S., Schioldan, A.G. Marco, M., Gregersen, S., Hermansen, K., 2018. Impact of diet-modulated butyrate production on intestinal barrier function and inflammation-a review. *Nutrients*. 10, 1-19. https://doi.org/10.3390/nu10101499
- Koo, B.S., Baek, S.H., Kim, G., Hwang, E. ha, Oh, H., Son, Y., Lim, K.S., Kang, P., Lee, H.Y., Jeong, K.J., Kim, Y.H., Villinger, F., Hong, J.J., 2019a. Idiopathic chronic diarrhea associated with dysbiosis in a captive cynomolgus macaque (*Macaca fascicularis*). J. Med. Primatol. 49, 56-59. https:// doi.org/10.1111/jmp.12447

- Koo, B., Hwang, E., Kim, G., Oh, H., Son, Y., Lee, D., Lim, K. S., Kang, P., Lee, S., Lee, H., Jeong, K., Lee, Y., Baek, S. H., Jeon, C., Park, S., Kim, Y., Huh, J., Jin, Y. B., Kim, S., Koo, B., 2019b. Evaluation of fecal microbiomes associated with obesity in captive cynomolgus monkeys (*Macaca fascicularis*). J. Vet. Sci. 20, 1-12. https://doi.org/10.4142/ jvs.2019.20.e19
- Kyes, R.C., 1993. Survey of the long-tailed macaques introduced onto Tinjil Island, Indonesia. Am. J. Primatol. 31, 77-83. https://doi.org/10.1002/ajp.1350310108
- Liedigk, R., Kolleck, J., Böker, K.O., Meijaard, E., Md-Zain, B. M., Abdul-Latiff, M.A.B., Ampeng, A., Lakim, M., Abdul-Patah, P., Tosi, A.J., Brameier, M., Zinner, D., Roos, C., 2015. Mitogenomic phylogeny of the common longtailed macaque (*Macaca fascicularis fascicularis*). *BMC Genomics*. 16, 1-11. https://doi.org/10.1186/s12864-015-1437-0
- Linares-Pastén, P.A., Hero, J.S., Pisa, J.H., Teixeira, C., Nyman, M., Adlercreutz, Martinez, M.A., Karlsson, E.N., 2021. Novel xylan-degrading enzymes from polysaccharide utilizing loci of *Prevotella copri* DSM18205. *Glycobiology*. 31, 1330-1349. https://doi.org/10.1093/glycob/cwab056
- Louis, P., and Flint, H.J., 2016. Formation of propionate and butyrate by the human colonic microbiota-a review. *Environ. Microbiol.* 19, 29-41. https://doi.org/10.1111/1462-2920.13589
- Lugano, S.D., Nyerere, K.A., Kariuki, W.K., Samuel, K., Joseph, K., Apondi, O.J., 2018. Gastrointestinal microbial flora in wild and captive olive baboons (*Papio anubis*). *Am. J. Infect. Dis.* 6, 30-37. http://dx.doi.org/10.12691/ajidm-6-1-5
- Martin, A.M., Sun, E.W., Rogers, G.B., Keating, D.J., 2019. The influence of the gut microbiome on host metabolism through the regulation of gut hormone release. *Front Physiol.* 10, 1-11. https://doi.org/10.3389/fphys.2019.00428
- McBurney, M.I., Davis, C., Fraser, C.M., Schneeman, B.O., Huttenhower, C., Verbeke, K., Walter, J., Latulippe, M.E., 2019. Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions. J. Nutr. 149, 1882-1895. https://doi. org/10.1093%2Fjn%2Fnxz154
- McKenzie, V.J., Song, S.J., Delsuc, F., Prest, T.L., Oliverio, A.M., Korpita, T.M., Alexiev, A., Amato, K.R., Metcalf, J.L., Kowalewski, M., Avenant, N.L., Link, A., Di Fiore, A., Orlando, A.S., Feh, C., Orlando, L., Mendelson, J.R., Sanders, J., Knight, R., 2017. The effects of captivity on the mammalian gut microbiome. *Integr. Comp. Biol.* 57, 690-704. https://doi.org/10.1093/icb/icx090
- Milton, K., 1999. Nutritional characteristics of wild primate foods: do the diets of our closest living relatives have lessons for us ?. *Nutrition*. 15, 488-98. https://doi.org/10.1016/S0899-9007(99)00078-7

- Newman, T.M., Shively, C.A., Register, T.C., Appt, S.E., Yadav, H., Colwell, R.R., Fanelli, B., Dadlani, M., Graubics, K., Nguyen, U.T., Ramamoorthy, S., Uberseder, B., Clear, K.Y.J., Wilson, A.S., Reeves, K.D., Chappell, M.C., Tooze, J.A., Cook, K.L., 2021. Diet, obesity, and the gut microbiome as determinants modulating metabolic outcomes in a nonhuman primate model. *Microbiome*. 9, 1-17. https://doi. org/10.1186/s40168-021-01069-y
- Perwitasari-Farajallah, D., Sawitri, H.I., Thiyana, S., Abimanyu, T.L., Iskandar, E., Darusman, H.S., 2023. Analyses of vegetation used by long-tailed macaque (*Macaca fascicularis* Raffles 1821) in Tinjil Island. J. Trop. Biodiveres. Biotechnol. 8, 1-10. https://doi.org/10.22146/jtbb.70739
- Plantain, M.L., Afolayan, A.J., 2019. Comparative evaluation of the nutritive, mineral, and antinutritive composition of *Musa* sinensis L. (banana) and *Musa paradisiaca* L. (plantain) fruit compartments. *Plants*. 8, 1-14. https://doi.org/10.3390/ plants8120598
- Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G.A.D., Gasbarrini, A., Mele, M.C., 2019. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases-a review. *Microorganisms*. 7, 1-22. https://doi.org/10.3390/ microorganisms7010014
- Rodrigues, N.R., Barbosa, J.L., Barbosa, M.I.M.J., 2016. Determination of physico-chemical composition, nutritional facts and technological quality of organic orange and purple-fleshed sweet potatoes and its flours. *Int Food Res* J. 23, 2071-78.
- Samuel, B.S., Shaito, A., Motoike, T., Rey, F.E., Backhed, F., Manchester, J.K., Hammer, R.E., Williams, S.C., Crowley, J., Yanagisawa, M., Gordon, J.I., 2008. Effects of the gut microbiota on host adiposity are modulated by the shortchain fatty-acid binding G protein-coupled receptor. *Proc Natl Acad Sci.* 105, 16767-16772. https://doi.org/10.1073/ pnas.0808567105
- Santoso, N., 1996. Analisis habitat dan potensi pakan monyet ekor panjang (Macaca fascicularis, Raffles) di Pulau Tinjil. Media Konservasi. 5, 5-9.
- Sawaswong, V., Praianantathavorn, K., Chanchaem, P., Khamwut, A., Kemthong, T., Hamada, Y., Malaivijitnond, S., Payungporn, S., 2021. Comparative analysis of oral-gut microbiota between captive and wild long-tailed macaque in Thailand. *Sci. Rep.* 11, 1-13. https://doi.org/10.1038/ s41598-021-93779-4
- Sawaswong, V., Chanchaem, P., Kemthong, T., Warit, S., Chaiprasert, A., Malaivijitnond, S., Payungporn, S., 2023. Alteration of gut microbiota in wild-borne long-tailed macaques after 1year being housed in hygienic captivity. *Sci. Rep.* 13, 1-13. https://doi.org/10.1038/s41598-023-33163-6

- Schwitzer, C., Polowinsky, S.W., and Solman, C., 2008. Fruits as foods-common misconceptions about frugivory. *Zoo Animal Nutrition*. 4, 131-168.
- Sha, J.C.M., Hanya, G., 2013. Diet, activity, habitat use, and ranging of two neighboring groups of food-enhanced long-tailed macaques (*Macaca fascicularis*). Am. J. Primatol. 75, 581-592. https://doi.org/10.1002/ajp.22137
- Tian, P., Gao, J., Liang, L., Cui, B., Hu, Q., Zhou, W., Li, B., Liu, Y., Chen, T., Rao, J., Wei, H., 2022. Fecal microbiota transplantation could improve chronic diarrhea in cynomolgus monkey by alleviating inflammation and modulating gut microbiota. *Biomedicines*. 10, 1-16. https:// doi.org/10.3390%2Fbiomedicines10123016
- Turnbaugh, P.J., Gordon, J.I., 2009. The core gut microbiome, energy balance and obesity. J. Physiol. 587, 4153-4158. https://doi.org/10.1113/jphysiol.2009.174136
- Wills, M.O., Shields-Cutler, R.R., Brunmeier, E., Weissenborn, M., Murphy, T., Knights, D., Johnson, T.J., Clayton, J.B., 2022. Host species and captivity distinguish the microbiome compositions of a diverse zoo-resident non-human primate population. *Diversity*. 14, 1-16. https://doi.org/10.3390/ d14090715
- Yeager, C.P., 1996. Feeding ecology of the long-tailed macaque (Macacafascicularis) in Kalimantan Tengah, Indonesia. Int. J. Primatol. 17, 51-62. https://doi.org/10.1007/BF02696158
- Yin L., Wan, Y.D., Pan, X.T., Zhou, C.Y., Lin, N., Ma, C.T., Yao, J., Su, Z., Wan, C., Yu, Y.W., Zhu, R.X., 2019. Association between gut bacterial diversity and mortality in septic shock patients: a cohort study. *Med. Sci. Monit.* 25, 7376-7382. https://doi.org/10.12659%2FMSM.916808
- Ying, C., Siao, Y., Chen, W., Chen, Y., Chen, S., Chen, Y., Hsu, J., 2022. Host species and habitats shape the bacterial community of gut microbiota of three non-human primates: Siamangs, white-handed gibbons, and Bornean orangutans. *Front. Microbiol.* 13, 1-20. https://doi.org/10.3389/ fmicb.2022.920190
- Zijlmans, D.G.M., Maaskant, A., Louwerse, A.L., Sterck, E.H.M., Langermans, J.A.M., 2022. Overweight management through mild caloric restriction in multigenerational longtailed macaque breeding groups. *Vet. Sci.* 9, 1-15. https:// doi.org/10.3390%2Fvetsci9060262



# **Supplementary Materials**

Figure S1. Tinjil Island, natural breeding facility of the primate research centre, located in the Southern Part of the Province of Banten



Figure S2. Group (GC\_Dramaga) (A) and individual (IC\_Dramaga) (B) cages of captive long tailed macaques in Dramaga breeding facilities, Primate Research Center, IPB University



Figure S3. Fecal samples of captive and semi-wild Long-tailed macaques demonstrating normal fecal consistency. (A) Captive long-tailed macaques in group cage (GC\_Dramaga), (B) Captive long-tailed macaques in individual cage (IC\_Dramaga), and (C) Semi-wild long-tailed macaques in Tinjil Island (SW\_Tinjil)



Figure S4. Wild plants consumed by semi-wild long-tailed macaques in Tinjil Island, including (A) Intsia bijuga, (B) Sterculia foetida, (C) Eugenia cymosa Lam., (D) Dysoxylum amooroides Miq



Figure S5. Relative abundance of the top 10 dominant gut bacteria phyla in captive (IC\_Dramaga and GC\_Dramaga) and semi wild (SW\_Tinjil) long-tailed macaques. The headmap displays all identified bacteria phyla found in each sample



Figure S6. Relative abundance of the top 10 dominant gut bacteria family in captive (IC\_Dramaga and GC\_Dramaga) and semi wild (SW\_Tinjil) long-tailed macaques. The headmap displays all identified bacteria family found in each sample



Figure S7. Relative abundance of the top 10 dominant gut bacteria genera in captive (IC\_Dramaga and GC\_Dramaga) and semi wild (SW\_Tinjil) long-tailed macaques. The headmap displays all identified bacteria genera found in each sample