

Impact of social media-driven feeding practices on the gut microbiome of captive Asian elephants (*Elephas maximus*) in Surin, Thailand

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Abstract

This study investigated the effects of social media-driven live-streaming dietary supplementation on the gastrointestinal microbial community of captive Asian elephants (*Elephas maximus*) at the Elephant Kingdom, Surin Province, Thailand. A hierarchical two-tier observational design was used: Tier 1 compared a minimal-supplementation control group (Group H, n = 6) with a frequent heterogeneous-supplementation group (Group L, n = 18); Tier 2 compared, within Group L, low-frequency (Group A, ≤ 3 sessions per week, n = 7) and high-frequency (Group B, > 3 sessions per week, n = 11) live-streaming sub-groups. Faecal microbial profiles were obtained by 16S rRNA gene (V3-V4) sequencing on an Illumina NovaSeq platform. Alpha diversity was tested by pairwise Wilcoxon rank-sum (Mann-Whitney U) tests; beta diversity by PERMANOVA on Bray-Curtis, weighted UniFrac and unweighted UniFrac distances; differential abundance by Metastats with Benjamini-Hochberg FDR correction and by LEfSe biomarker discovery. The community was dominated by Firmicutes (54.80%), Bacteroidota (26.30%), Verrucomicrobiota (5.28%), Actinobacteriota (3.63%), and Spirochaetota (3.47%). Alpha diversity did not differ between Groups H and L or between Groups A and B (all $P > 0.05$), and the dietary contrast in beta diversity was not statistically significant (Bray-Curtis PERMANOVA $R^2 = 0.053$, $P = 0.092$). Caldisericota was the only phylum to retain significance after FDR correction, being detected exclusively in Group H ($Q = 0.022$); LEfSe additionally identified Prevotellaceae as enriched in Group L (LDA = 4.17). These findings indicate that the captive Asian elephant gut microbiome is largely resilient at the community level to social media-driven dietary supplementation, while specific lineage-level signals — notably Caldisericota and Prevotellaceae — distinguish the dietary groups and warrant confirmation in larger longitudinal and functionally resolved studies.

Keywords: 16S rRNA sequencing, Asian elephant, Caldisericota, dietary fiber, gut microbiome, social media

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Received February 3, 2026

Accepted July 2, 2026

<https://doi.org/10.56808/2985-1130.4014>

Introduction

The health and physiological balance of mammals, including the Asian elephant (*Elephas maximus*), are fundamentally linked to the complex ecosystem of the gut microbiome (Feng *et al.*, 2023). In captive elephants, this microbial community is particularly sensitive, with its composition and diversity shaped by factors like diet, age, and living conditions (Klinhom *et al.*, 2023, 2025; Klinsawat *et al.*, 2023). The diet that sustains these animals is now at the center of a growing conflict between the economic survival of their human caretakers and the physiological needs of the elephants, a problem amplified by the rise of social media. Historically, captive elephants in Thailand were integral to industries such as logging. However, the nationwide logging ban in the late 20th century displaced thousands of elephants and their owners, pushing them into the tourism sector (Bansiddhi *et al.*, 2020). This transition created immense financial pressure, as the cost of care remained high while income became less certain. The recent COVID-19 pandemic devastated the tourism industry, leaving many elephant owners with no viable income and struggling to provide for their animals. This economic strain is acutely observed in major elephant conservation hubs, such as the Elephant Kingdom Project in Surin Province. As a primary residence for a large population of captive elephants displaced from the tourism sector, this site has become a focal point where owners have increasingly adapted to these financial challenges through digital engagement.

In a desperate bid for survival, many owners have turned to the digital world, using social media platforms like Facebook Live to connect with a global audience and solicit donations. A popular fundraising tactic involves viewers paying to have various low-fiber, high-sugar supplementary foods fed to the elephants on-screen. While this novel strategy provides a crucial financial lifeline, it has introduced a significant, unintended threat to elephant health. These social media-driven feedings often result in diets that are high in sugar and low in fiber, a stark departure from the high-fiber forage essential for their digestive health (Romain *et al.*, 2014). Moreover, the frequency and duration of these digital activities may directly determine the cumulative volume of low-fiber supplements consumed, where more frequent live-streaming sessions may lead to a higher intake of these non-traditional foods and potentially exacerbate gut health issues. Scientific literature underscores the critical role of dietary fiber in maintaining a healthy gut, promoting microbial diversity and metabolic well-being (Zhu *et al.*, 2015; Keady *et al.*, 2021; Zhang *et al.*, 2022). Conversely, low-fiber diets can lead to gut dysbiosis (Chen *et al.*, 2019), which has been linked to anthropogenic pressures and is associated with serious health issues in elephants (Klinhom *et al.*, 2025).

While previous studies have examined the gut microbiomes of captive elephants under various conditions (Keady *et al.*, 2021; Klinhom *et al.*, 2023; Klinsawat *et al.*, 2023), a significant knowledge gap exists regarding the specific consequences of these emerging, low-fiber feeding practices driven by social

media. The effects of a diet composed largely of low-fiber, high-sugar supplements (e.g., bananas, sugarcane, and various seasonal fruits), as well as the influence of feeding frequency during these digital interactions, remain largely uninvestigated. This study aims to address this gap by examining how these social media-influenced diets impact the gut microbiome of captive Asian elephants at the Elephant Kingdom in Surin Province. To achieve this, 16S rRNA gene sequencing was utilized to assess Alpha and Beta diversity, precisely quantifying shifts in microbial richness, evenness, and overall community structure associated with these novel feeding practices. By clarifying these microbial shifts, this research seeks to inform evidence-based management strategies that secure both elephant welfare and owner livelihoods.

Materials and Methods

Study design, sample collection, and dietary information and group characteristics: This study investigated the effects of dietary supplementation associated with social media-driven live-streaming activities on the gastrointestinal microbial community of captive Asian elephants at the Elephant Kingdom, Surin Province, Thailand. The procedures used in this study have been approved by Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU-011-057/2025). This study investigated the impact of low-fiber diets (i.e., heterogeneous mixed-composition diets), associated with live-streaming activities on social media, on the gastrointestinal microbiome of captive elephants at the Elephant Kingdom, Surin Province. Fecal samples were collected from a total of 24 elephants between August and September 2024. This study followed a hierarchical (nested) observational design with two pre-specified hypotheses: (i) dietary-supplementation effect of Group H (minimal supplementation, ≤ 1 occasion per week, $\sim <1\%$ of weekly dry-matter intake of low fiber diets, $n=6$) vs Group L (frequent heterogeneous supplementation associated with social media live-streaming, $n=18$); and (ii) live-streaming-frequency effect within Group L – Group A (≤ 3 sessions/week, $\sim 3\text{--}5\%$ of daily dry-matter intake of low fiber diets, $n=7$) vs Group B (>3 sessions/week, $\sim >5\%$ of daily dry-matter intake of low fiber diets, $n=11$). Each tier was analyzed independently to avoid conflation of dietary and behavioral effects. Three-group simultaneous tests were not performed. Comparative analysis of the gut microbiome profiles was conducted across these groups to determine the physiological consequences of these feeding practices.

All elephants were maintained under a standardized husbandry protocol with comparable physical activity, housing, and forage provision. The forage base (Napier grass, pineapple stalks, tree branches) was provided ad libitum during a daily 3–4-hour morning free-foraging period and was continuously available within the covered pens. Supplementary feeding events were monitored and recorded by caretakers in daily logs that captured the type, approximate amount, and timing of supplements provided, together with the start and end time of each

live-streaming session. Structured interviews were conducted with the elephant caretakers/owners using a standardized questionnaire. It elicited information on: (i) the primary forage diet; (ii) supplementary feeding patterns (food types, frequency per week, and approximate cumulative quantity per day, in kilograms); (iii) live-streaming activities (sessions per week, duration per session, and food types provided during broadcasts); and (iv) general husbandry, including housing, watering, and daily activity. To reduce recall bias, caretaker responses were cross-validated against direct observation by the research team during site visits and against the caretakers' daily feeding logs. Interview data together with the caretakers' daily feeding logs were used solely as the operational criterion for group classification (H, A, and B) and were not used as covariates in any inferential analysis.

Fresh fecal samples were collected from 24 captive elephants immediately after defecation using disposable rubber gloves and were placed in sterile zip-lock bags. Samples were maintained at 4 °C on ice during transport and were delivered to the laboratory of the Faculty of Veterinary Sciences, Mahasarakham University, within a maximum of 6 hours from collection. Upon arrival, each sample was homogenized within its sealed collection bag using a sterile spatula to mitigate the known spatial heterogeneity of microbial distribution within fecal material. Two-gram aliquots were then transferred into sterile microcentrifuge tubes, sealed with paraffin tape, and stored at -20 °C until DNA extraction.

DNA extraction and quality control: Metagenomic DNA was extracted from the fecal samples using commercial kits, following the manufacturers' protocols (PureLink Microbiome DNA Purification Kit, Invitrogen, Thermo Fisher Scientific, USA). The concentration and purity of the DNA were measured with a microvolume spectrophotometer (NanoDrop™ 2000/2000c Spectrophotometer, Thermo Fisher Scientific, USA). To ensure optimal DNA purity and concentration, a standard A260/A280 ratio of 1.7 to 1.9 with at least 5 ng/μl was employed (Malaluang *et al.*, 2024).

16S rRNA gene sequencing: The 16S rRNA gene sequencing and subsequent bioinformatic analyses were performed (Beijing Tsingke Biotech Co., Ltd, Beijing, China). Briefly, the V3-V4 hypervariable regions of the 16S rRNA gene were amplified using specific primers: Forward (5'-ACTCCTACGGGAGGCAGCA-3') and Reverse (5'-GGACTACHVGGGTWTCTAAT-3') (Accession number: PRJNA1130356). The PCR amplification was conducted in a 25 μl reaction containing 12.5 μl of 2' KAPA HiFi HotStart ReadyMix (Thermo Fisher Scientific, USA), 5 μl of forward primer, 5 μl of reverse primer, and 3 μl of genomic DNA template (5–10 ng). The thermal cycling conditions were initiated with a denaturation step at 95 °C for three minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at

72 °C for 30 seconds, with a final extension at 72 °C for five minutes.

The amplicons were used to construct sequencing libraries according to the standard Illumina protocol. Library quality and concentration were validated using 2100 Bioanalyzer (Agilent, CA, USA) before sequencing on the Illumina NovaSeq 6000 platform (Illumina, CA, USA). Raw reads were filtered for quality using Trimmomatic v0.33 (Bolger *et al.*, 2014), and primer sequences were removed using cutadapt 1.9.1 (Martin, 2011). High-quality reads were subsequently processed for denoising and chimera removal using the DADA2 pipeline (Callahan *et al.*, 2016) to generate non-chimeric Amplicon Sequence Variants (ASVs). Taxonomic annotation was then executed against the Silva 138 database. This comprehensive approach ensured high data quality and an accurate taxonomic profile of the elephant gastrointestinal microbiome.

Statistical and bioinformatics analysis: Statistical and bioinformatics analysis of the 16S rRNA gene sequences was performed using the QIIME2 pipeline (Bolyen *et al.*, 2019), utilizing the DADA2 plugin (Callahan *et al.*, 2016) for denoising and generating Amplicon Sequence Variants (ASVs). Taxonomic annotation was executed using a BLAST+ Bayesian classifier against the Silva database (version 138) (Quast *et al.*, 2013). Microbial composition at the phylum and genus levels was determined, with results expressed as percentage relative abundance. Alpha diversity, representing within-sample richness and evenness, was assessed using Chao1, Shannon, Simpson, ACE, and PD whole-tree (Faith's phylogenetic diversity) indices, as previously described (Malaluang *et al.*, 2024), with statistical differences between groups evaluated using the non-parametric Wilcoxon rank-sum (Mann-Whitney U) test, applied pairwise to the two pre-specified hierarchical contrasts (H vs L and A vs B within Group L); the non-parametric test was chosen because per-group sample sizes are small (n = 6–18), under which the normality assumption of parametric tests cannot be reliably verified. To compare microbial community structures between groups (beta diversity), Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances was performed using the Phyloseq R package (McMurdie and Holmes, 2013), and statistical significance was determined using PERMANOVA (Malaluang *et al.*, 2024). Differential abundance of microbial taxa across groups was analyzed using the Metastats software (White *et al.*, 2009), employing a two-sample t-test coupled with a permutation test. To account for multiple comparisons, p-values were adjusted using the False Discovery Rate (FDR) method. Statistical significance was predefined at a P-value < 0.05 and a q-value ≤ 0.05. Beta-diversity differences were further tested by Permutational Multivariate Analysis of Variance (PERMANOVA, 999 permutations) using the adonis2 implementation in the vegan R package (Oksanen *et al.*, 2022), applied separately to the two pre-specified hierarchical contrasts (H vs L and A vs B within Group L) on Bray-

Curtis, weighted UniFrac, and unweighted UniFrac distances; an ANOSIM test (Clarke, 1993) was reported alongside for the joint three-group display. To complement Metastats-based differential abundance and to identify discriminating biomarkers across taxonomic levels, Linear Discriminant Analysis Effect Size (LEfSe) was performed with a Kruskal-Wallis filter ($P < 0.05$) and an LDA score threshold of > 4 (Segata *et al.*, 2011). All taxon-level p-values reported in the Results are accompanied by their corresponding FDR-adjusted q-values.

Results

Dietary information and group characteristics: Based on structured interviews with elephant caretakers, the primary diet for all elephants consisted of Napier grass (*Pennisetum purpureum*), pineapple stalks, and tree branches. Significant dietary differences were observed in the supplementary feeding practices between the two categories. Group H was occasionally fed fruit supplementation, primarily bananas (approximately once per week). In contrast, Group L consumed a more diverse and frequent supply of heterogeneous supplements, including bananas, sugarcane, cucumbers, watermelons, jicama, pineapples, and corn. These supplements were provided daily, coinciding with social media live-

streaming sessions. While the exact quantity of these supplements could not be precisely quantified, the frequency and variety of heterogeneous diet intake in Group L were markedly higher than in Group H, establishing a clear distinction in feeding patterns between the control and live-streaming groups.

Taxonomic composition analysis: The purified DNA extracts from all 24 elephants were deemed suitable and proceeded to downstream library preparation and high-throughput sequencing data quality assessment. High-throughput sequencing of the 16S rRNA gene revealed substantial microbial diversity within the fecal microbiome of captive elephants. A total of 37,002 operational taxonomic units (OTUs) were identified across 24 samples, with individual richness ranging from a minimum of 1,076 to a maximum of 4,239 OTUs, indicating significant inter-individual variation. At the phylum level, the community was dominated by five major phyla: Firmicutes (54.80%), Bacteroidota (26.30%), Verrucomicrobiota (5.28%), Actinobacteriota (3.63%), and Spirochaetota (3.47%) (Table 1). At the genus level, the five most prevalent genera identified were *Sarcina* (9.05%), *Rikenellaceae_RC9_gut_group* (6.10%), *uncultured_rumen_bacterium* (5.47%), *Bacillus* (4.53%), and *unclassified_F082* (4.07%). These results reflect a complex and diverse gastrointestinal microbial ecosystem within the studied population.

Table 1 Relative abundance (%) of bacterial and archaeal phyla in the fecal microbiome of captive Asian elephants, presented overall and by group.

Phylum	Overall (%)	Group H (%)	Group A (%)	Group B (%)
Firmicutes	54.80	55.83 ± 10.97	57.57 ± 6.84	52.47 ± 10.09
Bacteroidota	26.30	24.31 ± 4.64	25.76 ± 2.89	27.73 ± 8.27
Verrucomicrobiota	5.28	5.07 ± 2.52	5.41 ± 2.56	5.31 ± 3.12
Actinobacteriota	3.63	5.12 ± 2.99	2.37 ± 1.19	3.63 ± 2.01
Spirochaetota	3.47	3.02 ± 2.44	3.72 ± 1.49	3.55 ± 2.01
Proteobacteria	2.67	2.51 ± 2.77	2.27 ± 3.00	3.02 ± 5.02
Fibrobacterota	1.07	0.80 ± 0.53	0.81 ± 0.51	1.38 ± 1.41
Synergistota	1.03	1.62 ± 0.72	0.56 ± 0.23	1.02 ± 0.71
unclassified Bacteria	0.48	0.60 ± 0.41	0.53 ± 0.26	0.39 ± 0.20
Cyanobacteria	0.43	0.28 ± 0.13	0.41 ± 0.11	0.52 ± 0.22
Desulfobacterota	0.33	0.21 ± 0.24	0.28 ± 0.27	0.42 ± 0.63
Patescibacteria	0.24	0.34 ± 0.33	0.17 ± 0.09	0.24 ± 0.20
Elusimicrobiota	0.068	0.090 ± 0.099	0.063 ± 0.094	0.059 ± 0.039
Chloroflexi	0.052	0.092 ± 0.146	0.015 ± 0.021	0.054 ± 0.121
Acidobacteriota	0.036	0.002 ± 0.003	0.003 ± 0.003	0.076 ± 0.249
Campylobacterota	0.027	0.048 ± 0.096	0.003 ± 0.004	0.031 ± 0.071
Fusobacteriota	0.012	0.021 ± 0.018	0.008 ± 0.006	0.009 ± 0.010
Myxococcota	0.011	0.002 ± 0.004	0.001 ± 0.003	0.022 ± 0.071
Other rare phyla (18 taxa)	0.036	0.018	0.012	0.060

Values for Groups H, A and B are mean ± standard deviation; the Overall column is the sample-size-weighted mean across all 24 individuals. Group H, minimal-supplementation control (n = 6); Group A, low-frequency live-streaming (n = 7); Group B, high-frequency live-streaming (n = 11). Phyla with an overall relative abundance $\geq 0.01\%$ are listed individually; the remaining 18 phyla, each $< 0.01\%$, are pooled in the final row. "0" denotes a phylum not detected; "<0.001" denotes a non-zero mean below 0.001%.

Alpha diversity analysis: Alpha diversity indices, including Chao1, Shannon, Simpson, ACE, and PD whole-tree (Faith's phylogenetic diversity), were

employed to evaluate the microbial community within Groups H, A, and B. Statistical comparisons were performed pairwise under the hierarchical design

using the Wilcoxon rank-sum (Mann-Whitney U) test. The dietary contrast (H vs L) revealed no significant differences across any of the indices (Shannon $P = 0.94$; Simpson $P = 0.63$; Chao1 $P = 0.63$; ACE $P = 0.63$; PD whole-tree $P = 0.22$). Likewise, the live-streaming-frequency contrast within Group L (A vs B) showed no significant differences (Shannon $P = 1.00$; Simpson $P = 0.28$; Chao1 $P = 0.54$; ACE $P = 0.54$; PD whole-tree $P = 0.40$). For species richness, the median Chao1 values for Groups H, A, and B were 1591.31, 1629.58, and 1579.52, respectively, suggesting a comparable level of taxonomic richness. Similarly, the overall species diversity, as reflected by the Shannon index, showed median values of 8.14 for Group H, 8.56 for Group A, and 8.16 for Group B. Furthermore, the Simpson index, which emphasizes species dominance, yielded median values of 0.984, 0.985, and 0.982 for the respective groups. Collectively, these findings indicate that the species richness, diversity, and dominance of the gastrointestinal microbial communities were not significantly altered by the different dietary and live-streaming conditions in this study population.

Beta diversity analysis: The beta diversity of the gastrointestinal microbial communities across the 24 fecal samples was visualized using Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances. Comparisons were conducted between Group H and Group L (Fig. 1A), as well as among Groups H, A, and B (Fig. 1B). Permutational Multivariate Analysis of Variance (PERMANOVA, 999 permutations) was performed separately for the two pre-specified hierarchical contrasts. For the dietary contrast (H vs L), no statistically significant difference in microbial community composition was detected (Bray-Curtis: $F = 1.23$, $R^2 = 0.053$, $P = 0.092$; weighted UniFrac: $P = 0.293$; unweighted UniFrac: $P = 0.088$). For descriptive purposes, the three-group display (H, A, B) returned a small but statistically detectable signal driven primarily by the within-L (A vs B) contrast (Bray-Curtis: $F = 1.27$, $R^2 = 0.108$, $P = 0.023$; unweighted UniFrac: $R^2 = 0.111$, $P = 0.038$; ANOSIM $R = 0.177$, $P = 0.015$). The grouping variables therefore explained only a small fraction ($\leq 11\%$) of the total microbial variation, and the dietary supplementation contrast itself did not reach statistical significance under the hierarchical analysis.

Differential abundance analysis: Comparative analysis of the fecal microbial composition between Group H and Group L using the Metastats software with False Discovery Rate (FDR) adjustment identified one phylum that survived multiple-testing correction. The phylum Caldisericota was detected exclusively within Group H and remained significant after FDR correction (raw $P = 6.06 \times 10^{-4}$; $q = 0.022$). The phylum Cyanobacteria showed a trend toward higher relative abundance in Group L (raw $P = 8.11 \times 10^{-3}$), but this signal did not survive FDR correction ($q = 0.150$) and is therefore reported here as suggestive rather than

statistically significant. No phylum reached $q \leq 0.05$ in the within-L (A vs B) comparison (Table 2). Complementary biomarker discovery using LEfSe (LDA threshold > 4) identified the family Prevotellaceae as enriched in Group L relative to Group H (LDA = 4.17, $P = 0.023$), and the genus Prevotellaceae_UCG_003 as enriched specifically in Group A (LDA = 4.10, $P = 0.008$). Taken together, the differential-abundance signal under proper multiple-testing control is modest: a single phylum-level taxon (Caldisericota) and a high-LDA, fiber-fermenting family (Prevotellaceae) distinguish the two dietary groups, while other phylum-level signals (including Cyanobacteria) are best interpreted as exploratory trends.

Discussion

The phylum-level analysis demonstrated a remarkably stable core microbial composition across all samples, characterized by the overwhelming dominance of Firmicutes and Bacteroidota. Firmicutes exhibited the highest relative abundance, ranging from 40.46% to 68.00%, followed by Bacteroidota at 27.60% to 50.32%. The consistent predominance of these two phyla underlines their role as the 'core microbiome' of Asian elephants, which is essential for the degradation of cellulose and complex carbohydrates derived from high-fiber diets (Klinsawat *et al.*, 2023). Other phyla, including Spirochaetota, Proteobacteria, Actinobacteria, Verrucomicrobiota, and Fibrobacterota, were present in low proportions. Collectively, the uniform distribution of these major phyla reflects a fundamental microbial community structure that is well-conserved among the studied population, which is typical in large hindgut fermenters. However, studies on captive elephants have reported variations in this phylum-level dominance, indicating that the gut community may shift in response to specific management practices (Li *et al.*, 2022; Thorel *et al.*, 2023). For instance, one study (Padur Sankaranarayanan *et al.*, 2025) observed a relatively reduced Firmicutes share (42.8%) in captive Asian elephants, with a high combined presence of Firmicutes, Proteobacteria, and Bacteroidota accounting for nearly 90% of the sequences. Such shifts frequently correlate with anthropogenic factors, including dietary changes, lifestyle gradients, and increased human contact (Zhang *et al.*, 2023; Padur Sankaranarayanan *et al.*, 2025). Specifically, a decrease in microbial diversity has been linked to the degree of captivity and the provision of heterogeneous diets, which can alter both the community membership and functional gene abundance (Thorel *et al.*, 2023; Zhang *et al.*, 2023). Our findings align with the perspective that while a core microbial framework remains, the relative dominance of major phyla is plastic and susceptible to the specific husbandry and dietary conditions of captive environments (Li *et al.*, 2022; Padur Sankaranarayanan *et al.*, 2025).

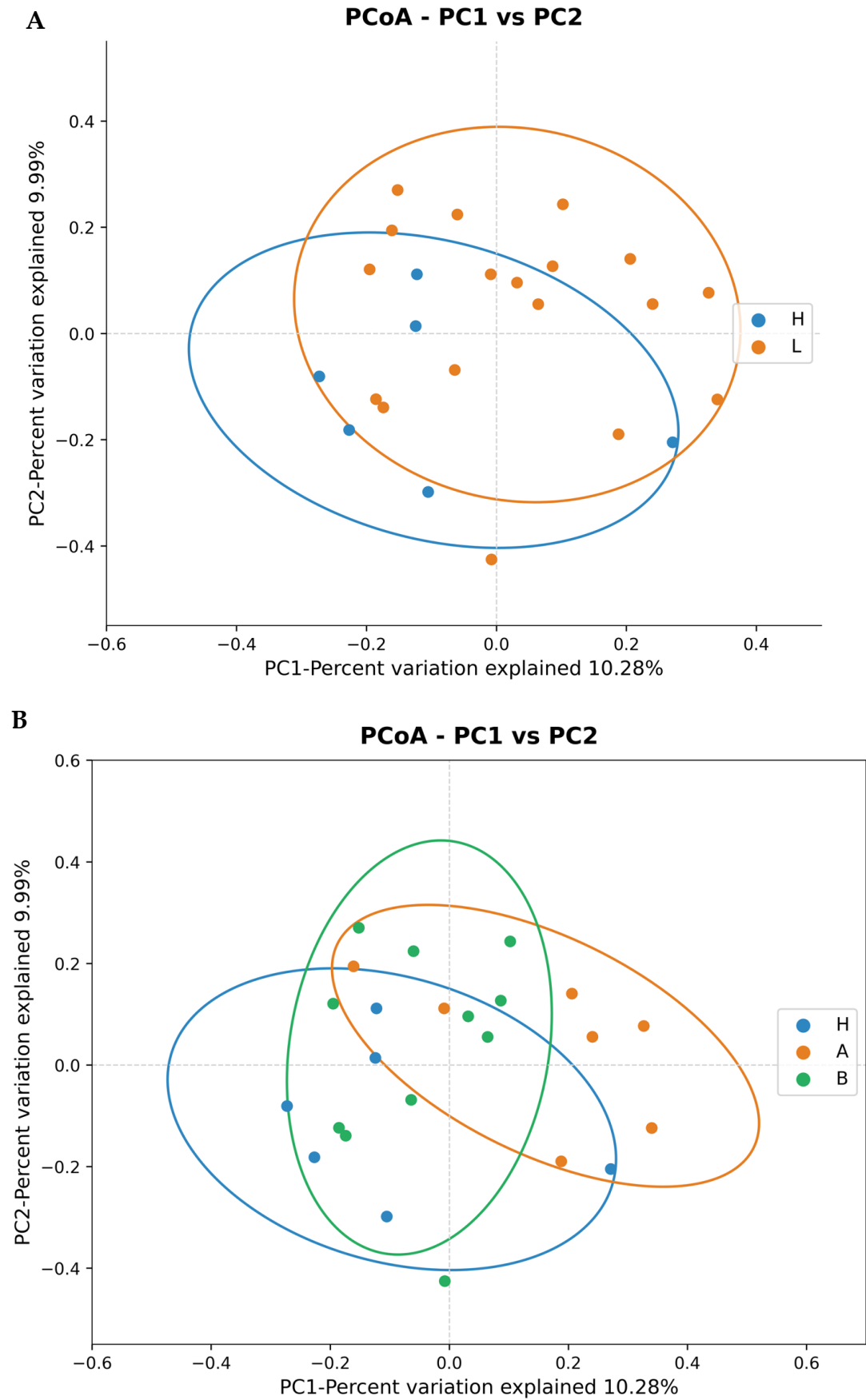


Figure 1 Principal Coordinates Analysis (PCoA) plots based on Bray-Curtis distances comparing the microbial community composition of captive elephant fecal samples: (A) Comparison between Group H and Group L; (B) Comparison among Groups H, A, and B.

Table 2 Differential relative abundance of bacterial and archaeal phyla between Group A and Group B, determined by Metastats analysis.

Phylum	Mean A (%)	Mean B (%)	P-value	Q-value (FDR)
Synergistota	0.56	1.02	0.033	0.477
Actinobacteriota	2.37	3.63	0.072	0.534
Cyanobacteria	0.41	0.52	0.128	0.676
Campylobacterota	0.003	0.031	0.176	0.676
Firmicutes	57.60	52.50	0.181	0.676
unclassified Bacteria	0.53	0.39	0.183	0.676
Fibrobacterota	0.81	1.38	0.201	0.676
Chloroflexi	0.015	0.054	0.292	0.799
Myxococcota	0.001	0.022	0.436	0.799
Acidobacteriota	0.003	0.076	0.462	0.799
Patescibacteria	0.18	0.24	0.561	0.799
Bacteroidota	25.80	27.70	0.645	0.823
Desulfobacterota	0.28	0.42	0.677	0.835
Fusobacteriota	0.008	0.009	0.785	0.897
Proteobacteria	2.27	3.02	0.800	0.897
Spirochaetota	3.72	3.55	0.883	0.961
Elusimicrobiota	0.063	0.059	0.921	0.966
Verrucomicrobiota	5.41	5.31	0.940	0.966
Caldiseriicota	0	0	1.000	1.000

Group A, low-frequency live-streaming (n = 7); Group B, high-frequency live-streaming (n = 11); both groups are nested within Group L. P- and Q-values are defined as in Table 2, and phyla are ordered by ascending P-value. No phylum differed significantly between Groups A and B after FDR correction (all Q > 0.05); in particular, Cyanobacteria did not differ between the two groups (P = 0.128, Q = 0.676). A further 18 phyla, each < 0.01% in overall relative abundance, were also tested with the same result.

More pronounced differences were observed upon deeper analysis at the genus level. While *Sarcina* was identified as the most abundant genus across all samples, significant variability was noted in multiple taxa. Among the cellulose-degrading taxa, *Treponema*, a key lignocellulose-degrading genus previously reported as enriched in captive Asian elephants under high-fiber diets (Li *et al.*, 2023; Thorel *et al.*, 2023), was also detected in our samples but at moderate relative abundance (~3.4 % on average). In the present study, the genus *Sarcina* exhibited a wide range of relative abundance, reaching a peak of 34.72% in a specific individual (I.9). The high prevalence of *Sarcina*, an anaerobic saccharolytic bacterium, may serve as an indicator of gastrointestinal dysbiosis or opportunistic fermentation, as this genus is frequently associated with acute gas production and abomasal disorders in various mammalian hosts (Vatn *et al.*, 2000; Makovska *et al.*, 2023). Similarly, although *Lysinibacillus* was present in relatively low proportions, it was consistently detected in every sample. While some *Lysinibacillus* strains possess potential for lignocellulose degradation, their consistent detection in our study is noteworthy as this genus is not typically regarded as a core taxon in healthy Asian elephants and has been specifically reported to increase during instances of gastrointestinal disorders such as impaction colic and diarrhea (Klinhom *et al.*, 2025). Such genus-level fluctuations suggest that individual-specific factors, including diet, environmental exposure, and husbandry management, may exert a

substantial influence on the gastrointestinal microbial composition. Furthermore, the presence of specific microbial lineages in captive cohorts has been interpreted as a signal of 'humanization' or anthropogenic contamination, often driven by prolonged human contact and environmental stressors inherent to captive management (Padur Sankaranarayanan *et al.*, 2025). These findings highlight the necessity for higher-resolution studies at the species or strain level to identify specific microbes susceptible to environmental and nutritional shifts. Such detailed insights will be crucial for better understanding the functional role of the microbiome in the health and nutrition of Asian elephants.

The analysis of Alpha diversity revealed no significant statistical differences among Groups H, A, and B. These findings indicate that the frequency of live-streaming sessions and the concurrent intake of heterogeneous dietary supplements did not significantly compromise the species richness, overall diversity, or species dominance within the gastrointestinal microbiome of the population of interest. This stability in Alpha diversity suggests that the core microbial population maintains a degree of resilience, where the introduction of supplementary heterogeneous foods does not lead to a drastic loss of microbial species or a collapse of community evenness within the timeframe of this study. Similar patterns of microbial resilience have been documented in other megaherbivores, such as black rhinoceroses and managed red deer, where supplemental feeding did

not significantly compromise alpha diversity indices despite compositional shifts (Gibson *et al.*, 2019; Zheng *et al.*, 2024).

Beta-diversity analysis was performed separately for the two pre-specified hierarchical contrasts. Under the dietary contrast (H vs L), PERMANOVA on Bray-Curtis distances did not detect a statistically significant difference in community composition ($R^2 = 0.053$, $P = 0.092$), and the same conclusion was reached on weighted ($P = 0.293$) and unweighted ($P = 0.088$) UniFrac distances. A small, statistically detectable signal emerged only when the three groups (H, A, B) were displayed jointly ($R^2 = 0.108$, $P = 0.023$ on Bray-Curtis; ANOSIM $R = 0.177$, $P = 0.015$), with the variance attributable mainly to the within-L (A vs B) contrast. Even in that joint display, the grouping variables collectively explained only ~11% of the total microbial variation. These results indicate that, at the community level, the dietary supplementation associated with live-streaming did not produce a statistically significant shift in beta diversity beyond the influence of live-streaming frequency within Group L. This suggests that while digital-mediated feeding practices are a contributing factor, they represent only a partial driver of microbiome shifts. The observed effect size is consistent with other wildlife studies, where specific dietary interventions often explain only a minor fraction (approximately 4–12%) of the total microbial variance, while larger proportions remain attributed to environmental context and individual host heterogeneity (Alberdi *et al.*, 2021; Xu *et al.*, 2025). Conversely, the remaining 89.2% of the variance remains unexplained by the factors studied, likely stemming from other unmeasured biological and environmental variables such as age, specific habitat conditions, baseline health status, and host genetics. Recent longitudinal and cross-sectional studies in Asian elephants have confirmed that age-linked transitions—from milk-associated taxa in infants to fibrolytic specialists in adults—along with distinct individualized microbial signatures, represent dominant drivers of community structure (Kambe *et al.*, 2020; Klinsawat *et al.*, 2023; Rojas *et al.*, 2023). These findings underscore the multifactorial nature of the elephant gut ecosystem and highlight the influence of individual variation beyond controlled dietary interventions.

An exploratory trend was observed for the phylum Cyanobacteria within the gastrointestinal tract of the study population. Cyanobacteria displayed a higher mean relative abundance in elephants consuming heterogeneous diets compared to those on the near-pure forage diet (raw $P = 0.008$), although this difference did not survive False Discovery Rate correction ($q = 0.150$) and should therefore be interpreted with caution. Notwithstanding this caveat, the directional pattern parallels observations in other species. For instance, Zeng *et al.* (2015) reported that high-weight Rex rabbits possessed a significantly greater proportion of the YS2 group—a lineage previously recognized as Cyanobacteria but recently reclassified as the non-photosynthetic class

'Melainabacteria'—within their hard feces. Metabolic analysis has demonstrated that this group possesses specialized functions, including obligate anaerobic fermentation, nitrogen fixation, and the synthesis of vitamins B and K, which may enhance animal digestion efficiency and support healthy growth. Taken together, these data suggest that Cyanobacteria (specifically Melainabacteria) may play a role in maintaining fermentative homeostasis in hindgut fermenters, although the direction of these shifts appears to be species-specific and dependent on nutritional status. In rabbits, this phylum is associated with improved energy harvest and weight gain, whereas in elephants, it may reflect a microbial adaptation to heterogeneous dietary intake. Specifically, these gut-resident bacteria potentially aid host nutrition under altered dietary regimes by compensating for nutritional deficits through vitamin biosynthesis and fermentative metabolism (Di Rienzi *et al.*, 2013). This discovery highlights the intricate relationship between dietary fiber and the microbiome of hindgut fermenters. Further investigation using functional metagenomics and metabolomics under controlled experimental conditions is warranted to elucidate the precise mechanisms underlying these microbial adaptations.

To our knowledge, this study represents the first report of the phylum Caldisericota within the gastrointestinal tract of a large hindgut-fermenting herbivore. The exclusive detection of Caldisericota in the high-fiber group (Group H) suggests a specialized niche, possibly linked to the degradation of complex organic matters. Metagenomic evidence from other anaerobic methanogenic consortia suggests that members of the Caldisericota (formerly candidate phylum OP5) may function as syntrophic partners (Mori *et al.*, 2009), potentially contributing to butyrate production and metabolic exchanges that support methanogenesis. Previously, Caldisericota have been predominantly documented in extreme environments, such as hot springs and oil seeps (Martinez *et al.*, 2019; Mori *et al.*, 2009). Consequently, our findings broaden the known ecological distribution of this phylum, suggesting its adaptability to the complex symbiotic ecosystems of megaherbivores. The absence of Caldisericota in elephants consuming heterogeneous diets further reinforces the concept that dietary composition is a critical determinant of microbial membership, where specific nutritional shifts can lead to the selective loss of unique microbial lineages.

It should be noted that this inference rests on a single phylum-level signal: among the other recognised fiber-fermenting taxa detected in our dataset (Fibrobacterota, Spirochaetota, family Ruminococcaceae), none exhibited a comparable Group-H-exclusive distribution at the FDR-adjusted level (Table 3). The Caldisericota observation should therefore be regarded as preliminary and hypothesis-generating, requiring confirmation by targeted shotgun-metagenomic or qPCR-based profiling of fibrolytic and methanogenic consortia in larger longitudinal cohorts.

Table 3 Differential relative abundance of bacterial and archaeal phyla between Group H and Group L, determined by Metastats analysis.

Phylum	Mean H (%)	Mean L (%)	P-value	Q-value (FDR)
Caldisericota	0.003	0	0.0006	0.022
Cyanobacteria	0.28	0.48	0.008	0.150
Synergistota	1.62	0.84	0.027	0.328
Fusobacteriota	0.021	0.009	0.128	0.806
Actinobacteriota	5.12	3.14	0.131	0.806
Bacteroidota	24.30	27.00	0.314	1.000
Fibrobacterota	0.80	1.16	0.354	1.000
Desulfobacterota	0.21	0.37	0.376	1.000
Acidobacteriota	0.002	0.048	0.434	1.000
Myxococcota	0.002	0.014	0.505	1.000
Patescibacteria	0.34	0.21	0.524	1.000
unclassified Bacteria	0.60	0.44	0.541	1.000
Chloroflexi	0.092	0.039	0.559	1.000
Campylobacterota	0.048	0.020	0.641	1.000
Elusimicrobiota	0.090	0.061	0.643	1.000
Spirochaetota	3.02	3.62	0.704	1.000
Firmicutes	55.80	54.50	0.842	1.000
Verrucomicrobiota	5.07	5.35	0.863	1.000
Proteobacteria	2.51	2.73	0.895	1.000

Group H, minimal-supplementation control (n = 6); Group L, combined live-streaming group (Groups A + B, n = 18). P-values were obtained from the Metastats two-sample test with permutation; Q-values are the corresponding Benjamini-Hochberg false discovery rate (FDR)-adjusted values. Phyla are ordered by ascending P-value. Values in bold denote the only phylum that remained significant after FDR correction ($Q \leq 0.05$). A further 18 phyla, each $< 0.01\%$ in overall relative abundance, were also tested and showed no significant difference between groups (all $Q > 0.05$).

Overall, the present observational data provide only limited evidence that the dietary supplementation associated with social-media live-streaming reshapes the gastrointestinal microbial community of captive elephants at the community level. It should be emphasised that this limited inferential strength is, in part, an unavoidable consequence of the study design rather than a shortcoming of the analysis. A controlled feeding trial – in which elephants would be experimentally assigned to a sustained low-fiber, high-sugar diet that is already recognised as detrimental to gastrointestinal health – would be neither ethically defensible nor practically feasible, as it would require knowingly exposing a long-lived, endangered species to a documented health risk for the purpose of experimentation. For this reason, a randomised, diet-controlled design could not be implemented, and the present study was necessarily observational, taking advantage of a naturally occurring divergence in feeding practices among elephant camps. While this approach safeguards animal welfare and reflects real-world husbandry conditions, it inherently constrains causal inference and contributes to the modest effect sizes reported here; the findings should therefore be read as associations rather than demonstrated causal effects. Alpha diversity did not differ between Group H and Group L, nor between Groups A and B under non-parametric Wilcoxon rank-sum testing, and beta diversity under the hierarchical H-vs-L contrast did not reach statistical significance (PERMANOVA Bray–

Curtis $P = 0.092$). A small, statistically detectable signal was observed only in the joint three-group display ($R^2 = 0.108$, $P = 0.023$), driven primarily by the within-L (A vs B) contrast rather than by the dietary contrast itself. Where compositional differences did emerge, they were largely at the lineage rather than community level, exemplified by the FDR-robust enrichment of Caldisericota in Group H ($q = 0.022$), the high-LDA enrichment of Prevotellaceae in Group L (LEfSe LDA = 4.17), and exploratory genus-level variation in taxa such as Sarcina and Lysinibacillus. Taken together, our findings indicate that the core diversity and community-level structure of the captive elephant gut microbiome are largely resilient to the dietary supplementation associated with social media-driven feeding, while a small number of specific microbial lineages – notably Caldisericota and Prevotellaceae – show a more sensitive, lineage-level response. The study therefore supports a measured rather than alarmist interpretation of the welfare implications of social-media-driven feeding under the conditions sampled, while providing a microbial-level baseline that motivates larger, longitudinal, and functionally resolved follow-up work.

This study has certain limitations that should be acknowledged. Notably, the precise daily nutrient intake, including fiber, sugar, and fat ratios, could not be quantified for each group. The feeding practices associated with social media live-streaming are inherently variable and uncontrolled, as the types and

quantities of supplementary foods are dictated in real-time by viewer donations during each broadcast session. Furthermore, the free-ranging management conditions at the Elephant Kingdom, where elephants forage on natural vegetation between live-streaming events, preclude accurate measurement of total daily intake. As such, our dietary characterization relied on structured interviews with caretakers to establish qualitative differences in feeding patterns rather than precise nutritional profiles. In addition, the per-group sample sizes were modest ($n = 6, 7$, and 11 for Groups H, A, and B, respectively), which constrains the statistical power of community-level tests and motivates our use of non-parametric Wilcoxon rank-sum testing for alpha diversity and of pairwise PERMANOVA contrasts for beta diversity. Under proper multiple-testing control (Benjamini-Hochberg FDR) (Benjamini and Hochberg, 1995), only a small number of differentially abundant taxa retained significance – most prominently *Caldisericota* and *Prevotellaceae* – whereas other phylum-level signals (including *Cyanobacteria*) did not survive correction and are reported here as exploratory trends rather than confirmed effects. The dietary contrast at the community level (H vs L) similarly did not reach statistical significance under PERMANOVA on Bray-Curtis, weighted UniFrac, or unweighted UniFrac distances; the small but statistically detectable signal observed in the joint three-group display was driven primarily by the within-L (A vs B) contrast. These observations should be interpreted as a baseline for hypothesis generation; replication in larger and longitudinal cohorts, with shotgun metagenomic and metabolomic characterization, will be required to confirm whether the lineage-level signals reported here reflect a stable functional response to social media-driven feeding. Despite this limitation, the study provides the first empirical evidence linking social media-driven feeding behaviors to measurable shifts in the gut microbial community structure of captive Asian elephants, establishing a critical baseline that can inform future controlled studies with detailed nutritional analyses.

Based on these findings, we recommend that elephant caretakers and activity organizers reconsider feeding practices during digital interactions. Prioritizing natural high-fiber forages while limiting high-sugar fruit supplements may help safeguard the long-term gastrointestinal health and welfare of captive elephants. Public education regarding appropriate elephant nutrition is also vital to foster community-wide support for these welfare standards. Future research should employ longitudinal designs to monitor the direct health outcomes of these microbial shifts and utilize higher taxonomic resolution (e.g., shotgun metagenomics) to further elucidate the functional roles of *Caldisericota* and *Prevotellaceae*. Additionally, investigating concurrent stressors, such as human-elephant interactions and environmental changes, would provide a more holistic understanding of the factors modulating the microbiome and overall health of this endangered species.

Acknowledgment

This research project was financially supported by Mahasarakham University.

Statement of author contributions: **Pongprecha Malaluang:** Conceptualization, Methodology, Project Administration, Writing – Original Draft, Writing – Review & Editing. **Nuttapon Bangkaew:** Investigation. **Jaruwan Chaikyachot:** Investigation. **Patinya Patikae:** Conceptualization, Methodology. **Nattaya Watwiengkam:** Conceptualization, Methodology. **Phimphanit Choklikitumnuey:** Formal Analysis, Visualization. **Thanaphat Phupanna:** Investigation, Data Curation. **Sudachanok Phowiset:** Investigation, Data Curation. **Prim Chuesaard:** Investigation, Data Curation. **Tarid Purisotayo:** Conceptualization, Methodology, Funding Acquisition, Project Administration, Supervision, Writing – Original Draft, Writing – Review & Editing.

Declaration of Artificial Intelligence Use: During the preparation of this work, the authors used Claude (Anthropic) for grammatical checking of the manuscript. The authors take full responsibility for the integrity, accuracy, and originality of the work.

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