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Nutritional profiling of stingless bee bread (Meliponini) across species and sites in Riau, Indonesia

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Abstract. *Suhesti E, Yelmiza, Fitriani D, Hadinoto. 2025. Nutritional profiling of stingless bee bread (Meliponini) across species and sites in Riau, Indonesia. Biodiversitas 26: 6151-6161.* Stingless bee bread is a nutrient-dense fermented pollen increasingly promoted as a functional food, yet Indonesian nutritional datasets remain scarce. This study characterizes proximate composition and vitamins A, B2, and E in bee bread from three stingless bee species (*Geniotrigona thoracica*, *Heterotrigona itama*, and *Trigona apicalis*) managed by smallholders at multiple sites in Riau, Indonesia. For each species × site combination, bee-bread pots were pooled to obtain a homogenized composite, which was summarized on a unified fresh-mass basis. Group differences among species and sites were evaluated using non-parametric tests. At a co-occurring site, *G. thoracica* consistently exhibited the highest levels of vitamins A, B_2, and E; *T. apicalis* showed the highest total fat and fat-derived energy; and *H. itama* combined relatively high total energy with elevated protein. Within *H. itama*, site effects were evident, with vitamins A and E peaking at specific locations and vitamin B_2 at another. These patterns resolve into three practical nutritional signatures, vitamin-oriented in *G. thoracica*, lipid-oriented in *T. apicalis*, and relatively stable energy or protein in *H. itama*. While the pooled composite design constrains colony-level inference, the species- and site-resolved ranges reported here provide preliminary nutritional baselines that can inform future work on quality control and biodiversity-aligned meliponiculture.

Keywords: Agroforestry, nutritional variability, proximate composition, smallholder meliponiculture, stingless bee bread

INTRODUCTION

Bee bread, a product of stingless bees, is formed through the fermentation of nectar and pollen, which leads to the production of organic acids and polysaccharides. This fermentation process lowers pH, stabilizes the matrix, and can enhance the availability of proteins, vitamins, and amino acids, as shown in recent studies on fermented bee products (Roman et al. 2016; Dranca et al. 2020; İspirli and Dertli 2021; Özkök et al. 2022; Aksoy et al. 2024; Ilie et al. 2024). In addition to macronutrients, bee bread also contains phenolics and flavonoids, which contribute to its antioxidant properties and health benefits, positioning it as a functional food (İspirli and Dertli 2021; Özkök et al. 2022; Aksoy et al. 2024; Ilie et al. 2024). This study focuses on establishing a nutritional baseline for bee bread from stingless bees in Riau by analyzing proximate composition and selected vitamins, excluding functional bioactivity metrics such as antioxidant capacity, phenolic and flavonoid profiles, alkaloids, and microbiome composition, which will be explored in a separate study.

Stingless beekeeping plays a crucial role in tropical agroforestry systems, supporting pollination of understory plants and fruit trees while offering smallholder farmers an additional source of income. The nutritional quality of bee bread reflects complex interactions between bees, host plants, and microbial communities. Its composition varies according to factors such as pollen spectra, site microclimate, microbial consortia, colony management, and foraging behavior, with the microbiome and fermentation processes transforming plant metabolites. Understanding this variability can strengthen the evidence base for standardized products and contribute to rural food security and public health (Luo et al. 2021; Suleiman et al. 2021; Ghosh et al. 2022; Keyvan et al. 2023; Cerqueira et al. 2024; Didaras et al. 2024; Li et al. 2024; Melia et al. 2024; Ng et al. 2024).

In Indonesia, despite the growing interest in stingless bee products, comprehensive datasets on bee bread are lacking. The country hosts high stingless bee diversity and expanding meliponiculture, but many studies focus more on honey or propolis rather than bee bread. Studies that measure proximate composition and vitamins using harmonized protocols are still rare. The discrepancies in methodology, including differences in mass reporting, moisture correction, extraction solvents, chromatographic conditions, and vitamin quantification, hinder the establishment of reliable nutritional ranges and obscure patterns related to species identity and site conditions (Bobiş et al. 2017; Urcan et al. 2017; Othman et al. 2019; Dranca et al. 2020; Erwan et al. 2021; Uslinawaty et al. 2023; Aksoy et al. 2024; Ilie et al. 2024).

This study aims to fill these gaps by profiling bee bread from three stingless bee species (*G. thoracica, H. itama,* and *T. apicalis*) maintained by smallholder farmers in Riau, Sumatra, Indonesia. Using standardized procedures, the study analyzes proximate parameters (moisture, ash, protein, fat, carbohydrates, and energy) and quantifies vitamins A, B2 (riboflavin), and E using validated chromatographic techniques. The measurements are conducted in triplicate, with appropriate quality assurance, and statistical analysis is performed using non-parametric tests to evaluate differences between species and sites. The novelty of this study lies in the integration of species and site-specific nutritional data within a consistent analytical framework, producing species-resolved and site-resolved nutritional baselines for bee bread.

The study addresses three main research questions and hypotheses. First, it explores whether there are consistent nutritional differences between species co-occurring at the same site, testing the hypothesis that bee bread nutritional profiles differ among species due to distinct pollen portfolios and fermentation dynamics. Second, it examines the site effect within *H. itama*, hypothesizing that nutritional components and vitamin concentrations differ among sites with varying forage sources and landscape contexts. Finally, the study investigates whether reporting on a unified fresh-mass basis with explicit statistical annotations can yield reliable reference ranges for bee bread, hypothesizing that uniform reporting can contribute to the establishment of quality standards once validated across seasons, colonies, and laboratories.

MATERIALS AND METHODS

Study site and sampling

Bee bread was collected from smallholder meliponiculture sites in Riau, Indonesia (Table 1, Figure 1), covering three focal species (*Geniotrigona thoracica*, *Heterotrigona itama*, and *Trigona apicalis*) to capture interspecific and inter-site variation. We focused on three meliponine species because they dominate smallholder meliponiculture in Riau and represent contrasting foraging ecologies. This taxonomic scope is appropriate for a provincial baseline but does not cover the broader Meliponini diversity across Indonesia. Sampling was conducted during the dry season under broadly comparable husbandry conditions to minimize temporal and management biases. The species-by-site design is summarized in Figure 2. To contextualize nutritional profiles, we summarized site-level microclimate (air temperature, relative humidity, wind speed, and light intensity) and forage-vegetation diversity (richness and H′). Site-level microclimate and forage-vegetation metrics for these apiaries were previously quantified in a companion environmental study; we reproduce the key microclimate and diversity summaries in Tables S1-S2 to provide ecological context for the present nutritional analysis. For each species at each site, mature bee-bread pellets from sealed pots were pooled into a single homogeneous composite for laboratory analysis. All measurements were performed as technical triplicates on this composite.

Table 1. Coordinates of stingless bee study sites in Riau, Indonesia

Site (Village)	Regency	Coordinates
Kuok	Kampar	0°18’44”N 100°56’53”E
Umban Sari	Pekanbaru	0°34’59”N 101°25’10”E
Merempan Hilir	Siak	0°48’14”N 101°57’40”E
Batu Rijal	Indragiri Hulu	0°30’59”S 101°57’21”E
Usul	Indragiri Hulu	0°43’55”S 102°30’01”E

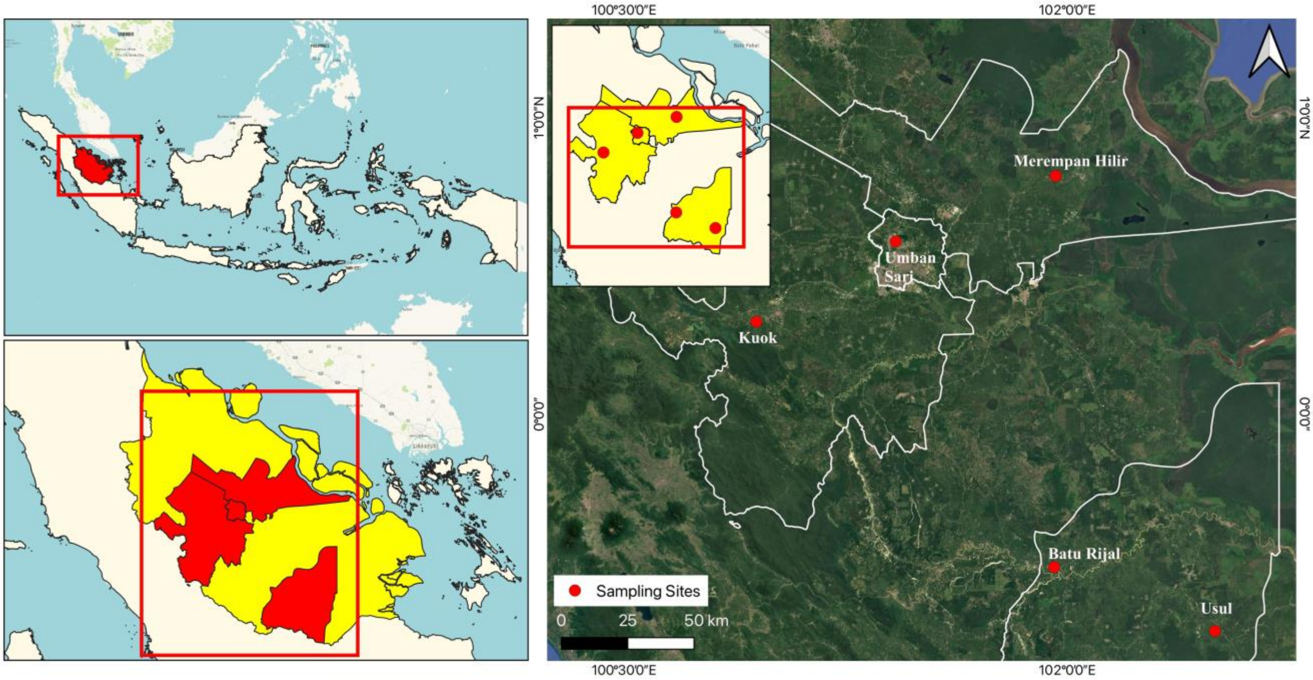

Figure 1. Locations of smallholder stingless-bee sites in Riau Province, Sumatra, Indonesia

Figure 2. A. Representative bee-bread pot during collection from a managed *Heterotrigona itama* colony. B. Labeled sterile containers of bee bread composites by species and site used for laboratory analysis

Sample preparation

Bee bread was aseptically collected into sterile containers, transported at 4-6°C, and processed within one week to minimize post-harvest changes. A single homogenized composite was prepared for each species at each site, as described in Study site and sampling. Laboratory assays followed accredited methods (PT Saraswanti Indo Genetech, ISO/IEC 17025). Detailed chromatographic and preparation parameters are summarized in Table S4.

Proximate composition

Proximate composition included moisture, ash, protein, fat, and carbohydrates by difference. Moisture was determined by controlled oven drying (Memmert UN55), ash by muffle incineration (Nabertherm L9/11), protein by Kjeldahl (Gerhardt Vapodest 50s, nitrogen-to-protein factor 6.25), and fat by solvent extraction (Buchi B-811), with carbohydrates calculated by difference. Essential procedural elements are summarized here; full analytical parameters for proximate assays are provided in Table S4.

Vitamin analysis

Riboflavin (vitamin B2) was quantified by HPLC using 0.1% phosphoric acid and acetonitrile as mobile phases at 0.30 mL min^{-1}, column temperature 40°C, injection volume 5 µL, diode-array scan 210-400 nm, with quantitation at 265 nm. Fat-soluble vitamins were analyzed on a Shimadzu LC-20AD with PDA and a C18 column under a water-methanol gradient (flow 1.5 mL min^{-1}, injection 20 µL), with quantitation at 325 nm (vitamin A) and 290 nm (vitamin E). Core chromatographic settings are summarized here; full parameters and validation criteria are provided in Table S4.

Quality control

Analysis was performed by an ISO/IEC 17025-accredited laboratory (PT Saraswanti Indo Genetech) using AOAC/SNI-conformant methods. Because the contracted laboratory does not release full validation sheets with client reports, we summarize typical validation criteria in Table S4, and dataset-specific QA metrics (triplicate precision and mass-balance closure) in Table S5. All reported datasets met these acceptance criteria (linearity thresholds, recovery ranges, and LOD/LOQ margins), ensuring that the detected concentrations are well above method detection limits.

Statistical analysis

Because each group comprised technical triplicates derived from a pooled composite (not independent biological replicates), we summarized data as median (IQR). We used non-parametric tests: Kruskal-Wallis for ≥3 groups and pairwise Mann-Whitney with Holm family-wise error control (α: 0.05). Shapiro-Wilk and Levene diagnostics are provided only for descriptive purposes; inferential decisions rely on the non-parametric framework. To complement p-values under small n, we report effect sizes (Cliff's delta) in Table S3. For the Siak site, proximate data were summarized as median (IQR) per species and analyzed using the same non-parametric framework (Kruskal-Wallis followed by pairwise Mann-Whitney with Holm correction).

RESULTS AND DISCUSSION

Proximate composition by location (*Heterotrigona itama*)

Across locations, median (IQR) summaries (Table 2) showed apparent numerical variation in several proximate parameters; however, non-parametric Kruskal-Wallis tests followed by pairwise Mann-Whitney tests with Holm correction did not detect statistically significant differences at α: 0.05 for any parameter. Given that the sample size at each location was limited to technical triplicates, we additionally report effect sizes (Cliff's delta) in the supplementary material. These effect sizes reveal enormous contrasts in moisture between Batu Rijal and the other locations, even though two-sided significance tests did not reach α: 0.05 under the small-n design. This pattern highlights the limited statistical power of the present design and underscores the need for biological (between-colony) replication in future work.

Interspecific contrasts at a co-occurring site

At the Siak site, median proximate profiles showed apparent numerical differences among stingless bee species (Table 3). Global Kruskal-Wallis tests indicated significant between-species variation in moisture, ash, total energy, protein, carbohydrates, total fat, and energy derived from fat (all $p<0.05$). However, pairwise Mann-Whitney comparisons with Holm correction did not identify any species pair as statistically distinct at α: 0.05, reflecting the small sample size (n: 3 technical replicates per species). Accordingly, the between-species contrasts in Table 3 are best interpreted as descriptive trends in median values rather than definitive pairwise separations.

Vitamin profiles across species and sites

At the co-occurring site in Siak, species-level contrasts revealed apparent differences in vitamin profiles among the three stingless bee taxa (Figure 3). *G. thoracica* showed the highest median levels of vitamins A, B2, and E, with narrow interquartile ranges indicating consistent enrichment across technical replicates. In contrast, *T. apicalis* contained virtually no detectable vitamins A and B_2 (median: 0 for both). Still, they retained moderate vitamin E concentrations, suggesting that this species may rely on floral resources that are relatively poor in carotenoids and riboflavin yet sufficient to supply lipophilic antioxidants. *H. itama* occupied an intermediate position: its bee bread exhibited substantially lower vitamin A than *G. thoracica*, but non-zero B2 and E levels with tight IQRs, pointing to a more balanced, although less vitamin-rich profile. Taken together, these patterns are consistent with the idea that species-specific foraging preferences and pollen-handling can generate distinct vitamin fingerprints even within a shared landscape context. However, this mechanism was not directly tested in the present design.

Within *Heterotrigona itama*, site-level contrasts were most pronounced for vitamin A, whose median concentrations ranged from 705.31 (9.37) µg·100 g^{-1} at Batu Rijal to 2864.91 (8.28) µg·100 g^{-1} at Usul, with Kuok also exhibiting elevated values. In contrast, Umban Sari and Merempan Hilir showed intermediate levels (Table 4). In comparison, vitamins B2 and E varied over a much narrower range, with all sites retaining detectable but relatively modest concentrations. Kruskal-Wallis tests confirmed significant overall site effects for all three vitamins ($p<0.05$), yet pairwise Mann-Whitney comparisons with Holm correction did not identify any site pair as statistically distinct at α: 0.05, reflecting the limited number of technical replicates per site. Accordingly, the cross-site gradients in median vitamin levels are best interpreted as descriptive patterns associated with differences in local environmental and management context, rather than as definitive causal effects of any single site attribute.

Discussion

Landscape context and field conditions

Local microclimate and vegetation structure were quantified as site-level context (Tables S1-S2), and given the composite sampling design, any associations with nutrient metrics are interpreted as descriptive/correlational, not causal. The interspecific and inter-site nutritional contrasts observed in Riau are consistent with smallholder agroforestry mosaics that provide heterogeneous floral resources and foraging windows. Taken together, these contextual covariates plausibly influence colony nutrition, in line with prior studies (Alaux et al. 2017; Donkersley et al. 2017, 2018; Martinello and Mutinelli 2021; Crone et al. 2022; Jorquera et al. 2023; Ilie et al. 2024).

Species-level nutritional differentiation

The three focal taxa showed clear nutritional signatures under shared field conditions: *Geniotrigona thoracica* displayed a vitamin-oriented profile, *Tetragonula apicalis* was lipid-oriented, and *Heterotrigona itama* combined relatively high total energy with elevated protein. These signatures synthesize measured contrasts in proximate composition and vitamin concentrations without repeating the numerical values reported in the tables and figures. Comparable species-linked differentiation in bee bread of stingless bees has been observed across regions, suggesting that species identity interacts with landscape context to structure nutritional profiles (Othman et al. 2019; Martinello and Mutinelli 2021; Jorquera et al. 2023; Ilie et al. 2024).

Table 2. Proximate composition of *Heterotrigona itama* of bee bread across five smallholder sites in Riau, Indonesia, expressed as median (IQR) (n: 3 technical replicates per site)

Location	Moisture (%)	Ash (%)	Total energy (kcal·100 g^{-1})	Protein (%)	Carbohydrate (%)	Total fat (%)	Energy from fat (kcal·100 g^{-1})
Umban Sari	28.19 (0.16)	1.59 (0.02)	308.48 (1.26)	12.70 (0.14)	52.02 (0.23)	5.52 (0.11)	49.68 (0.95)
Kuok	28.11 (0.11)	2.27 (0.03)	307.46 (1.09)	19.52 (0.25)	44.31 (0.22)	5.80 (0.10)	52.20 (0.95)
Batu Rijal	38.50 (0.16)	1.83 (0.00)	250.97 (0.80)	8.97 (0.09)	48.27 (0.20)	2.45 (0.04)	22.05 (0.36)
Usul	28.55 (0.15)	2.17 (0.02)	308.52 (0.92)	14.76 (0.09)	48.24 (0.04)	6.28 (0.08)	56.52 (0.72)
Merempan Hilir (Siak)	27.11 (0.19)	2.13 (0.03)	309.64 (1.23)	18.33 (0.15)	47.11 (0.30)	5.32 (0.07)	47.88 (0.63)

Note: Values are expressed as median (IQR) of technical triplicates per site. Differences among locations were tested using Kruskal-Wallis followed by pairwise Mann-Whitney tests with Holm correction (α: 0.05)

Table 3. Proximate composition of bee bread of stingless bees from three species at a co-occurring site in Siak (Merempan Hilir), expressed as median (IQR) (n: 3 technical replicates per species)

Species	Moisture (%)	Ash (%)	Total energy (kcal·100 g^{-1})	Protein (%)	Carbohydrate (%)	Total fat (%)	Energy from fat (kcal·100 g^{-1})
H. itama	27.11 (0.18)	2.13 (0.03)	309.64 (1.23)	18.33 (0.16)	47.11 (0.30)	5.32 (0.07)	47.88 (0.64)
G. thoracica	31.50 (0.05)	2.56 (0.01)	295.39 (0.17)	16.09 (0.11)	43.70 (0.32)	6.25 (0.10)	56.25 (1.00)
T. apicalis	32.48 (0.18)	1.77 (0.03)	301.05 (0.19)	16.24 (0.06)	41.91 (0.18)	7.61 (0.09)	68.44 (0.76)

Note: Values are expressed as median (IQR), where IQR is the interquartile range (Q3-Q1) of three technical replicates. Differences among groups were tested using Kruskal-Wallis followed by pairwise Mann-Whitney tests with Holm correction (α: 0.05)

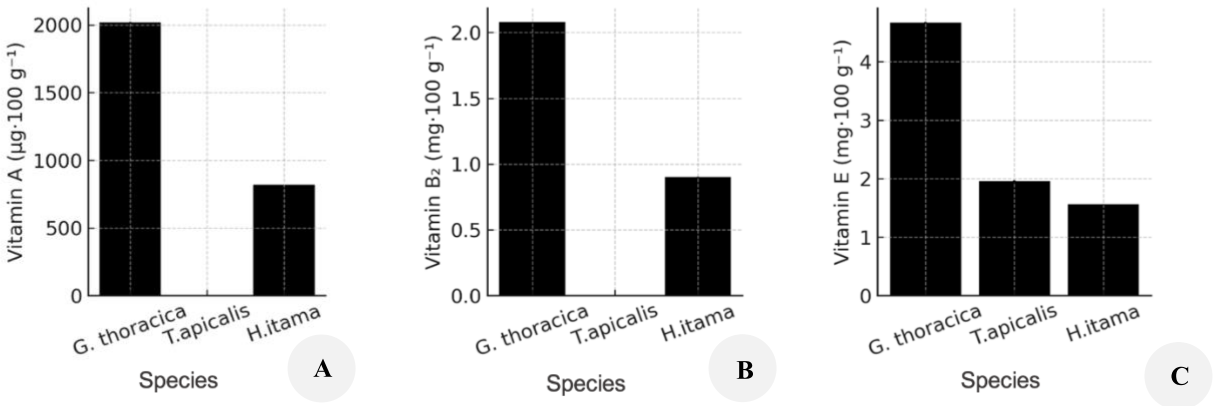

Figure 3. Median (IQR) of A. Vitamins A, B. Vitamin B_2, C. Vitamin E in bee bread of stingless bees by species at a co-occurring site (Siak). Bars show species-level medians from three technical replicates per species; error bars indicate the interquartile range (Q3-Q1). *Geniotrigona thoracica* exhibited the highest median concentrations of vitamins A, B_2, and E, whereas *Trigona apicalis* showed negligible levels of vitamins A and B_2 but moderate vitamin E contents, with *Heterotrigona itama* occupying an intermediate position

Table 4. Fat- and water-soluble vitamin content in *Heterotrigona itama* bee bread across different locations

Location	Vitamin A ($\mu g \cdot 100\ g^{-1}$)	Vit B_2 ($mg \cdot 100\ g^{-1}$)	Vitamin E ($mg \cdot 100\ g^{-1}$)
Umban Sari	1437.40 (1.22)	0.81 (0.01)	2.23 (0.00)
Kuok	2471.36 (15.23)	1.13 (0.01)	2.55 (0.04)
Batu Rijal	705.31 (9.37)	0.86 (0.00)	3.03 (0.03)
Usul	2864.91 (8.28)	0.90 (0.02)	3.43 (0.02)
Merempan Hilir (Siak)	818.25 (1.74)	0.90 (0.01)	1.56 (0.01)

Note: Kruskal-Wallis tests indicated significant overall location effects ($p<0.05$) for all vitamins; pairwise Mann-Whitney tests with Holm correction did not detect significant differences between locations at α: 0.05

Site effects within Heterotrigona itama

Within *H. itama*, site effects were evident. Vitamins A and E reached peak values at specific locations, whereas vitamin B2 peaked at another site (Table 4). We interpret these patterns conservatively as correlations with site context observed within the present design. Testing whether forest adjacency, pollen portfolios, nectar and resin sources, or colony management practices are the proximate drivers will require targeted measurements beyond the scope of this dataset (Donkersley et al. 2018; Crone et al. 2022).

Mechanistic interpretation: Forage composition and fermentation

Two complementary hypotheses plausibly connect field context to nutritional outcomes. Hypothesis 1 states that variation in pollen composition and nectar or resin inputs structures macronutrient and vitamin profiles through substrate availability; diverse and evenly distributed nectar and pollen sources supply precursors for carotenoids and tocopherols and for B vitamin cofactors (Colwell et al. 2017; Donkersley et al. 2017; Crone et al. 2022).

Hypothesis 2 states that fermentation dynamics and colony-associated microbiota modulate vitamin retention and transformation during bee bread maturation; lactic acid bacteria consortia can soften pollen walls, release bound phytochemicals, and protect or synthesize vitamins, shaping biochemical fingerprints that differ by species and site (Dranca et al. 2020; İspirli and Dertli 2021; Bakour et al. 2022; Ghosh et al. 2022; Çobanoğlu et al. 2023; Didaras et al. 2024). These literature-based mechanisms are consistent with the detected patterns and motivate follow-up using palynology and 16S or ITS profiling to test causality directly. These mechanistic explanations are hypothesis-based and were not directly tested here.

Cross-study comparison and reporting consistency

Variation among studies in proximate and vitamin values often reflects inconsistent reporting bases, for example, fresh weight versus dry weight, and differences in extraction protocols and chromatographic parameters (Bobiş et al. 2017; Othman et al. 2019; Dranca et al. 2020). Adopting unified reporting, a clear mass basis, consistent units, explicit moisture corrections, and shared calibration standards with transparent statistical marking will facilitate meta-analysis and allow differences to be attributed to biology rather than laboratory artefacts (Martinello and Mutinelli 2021; Jorquera et al. 2023; Ilie et al. 2024). In the Indonesian context, recent work on *H. itama* highlights its bioactive potential and opportunities for processing-aided value addition, such as encapsulation, which are relevant for stability and differentiated markets (Suleiman et al. 2021; Sari et al. 2024).

Site-level microclimate and forage profiles (Tables S1-S2) align with environmental windows reported to support Meliponini foraging in lowland tropics, which may partly explain between-site contrasts in nutrient levels observed here. A fuller treatment of these abiotic-floral gradients is presented in the companion study.

Limitations and implications

Contextual covariates were summarized at the site level (Tables S1-S2) and were not included as predictors in statistical models; any links to nutrient metrics are therefore descriptive rather than causal. This study did not conduct palynological profiling, so floral origins are inferred only from site-level vegetation context and should not be interpreted as definitive pollen composition. Our chemical profiling was also restricted to proximate composition and a small set of vitamins. We did not quantify functional bioactivity indices such as total phenolics, flavonoids, antioxidant capacity, or bee bread-associated microbiota. As a result, the nutritional baselines reported here cannot be directly translated into mechanistic explanations of health effects or shelf-life properties. They should instead be viewed as compositional benchmarks for bee bread of stingless bees under the sampled conditions. In addition, colony management practices were not standardized or systematically recorded across sites. Hive age, colony strength, and any supplementary feeding regimes likely differed among smallholder apiaries and may contribute to the observed variability in macronutrient and vitamin levels. The between-site contrasts reported here should therefore be interpreted as composite outcomes of environmental context and local husbandry rather than as effects of ecological conditions alone, and future studies should implement harmonized management protocols and quantitative recording of colony metrics to partition environmental and management contributions. Despite these constraints, the design enabled tractable comparisons across species and locations and provides an empirical baseline for follow-up studies incorporating biological (between-colony) replication. To enable robust attribution of side effects and to link compositional variation to functional properties, future work should integrate pollen microscopy or DNA metabarcoding, multi-season and multi-laboratory sampling, and joint measurement of stand structure, flowering phenology, and microclimatic conditions alongside chemical profiling, so that subsequent datasets can underpin more robust product standardization, labelling frameworks, and functional interpretation.

In conclusion, our findings provide a site-specific nutritional baseline for bee bread from stingless bees on smallholder farms in Riau, Indonesia. Species- and site-resolved profiles indicate that *G. thoracica* tends to produce vitamin-oriented bee bread, *T. apicalis* shows a more lipid-oriented profile, and *H. itama* maintains relatively stable energy and protein values across sites. These patterns offer an initial provincial snapshot that can inform early discussions on quality control, product development, and biodiversity-friendly meliponiculture planning, while remaining firmly descriptive. Given the pooled composite design, single-season sampling, and single-laboratory analysis, the ranges reported here should be regarded as preliminary, context-specific reference values rather than enforceable standards, certification thresholds, or definitive label claims. Priority next steps are to incorporate colony-level biological replication, multi-season and multi-laboratory sampling, and palynological and microbiome assays so that future datasets can underpin robust product standardization, labeling frameworks, and mechanistic understanding of bee bread functionality.

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Table S1. Site-level microclimate at stingless-bee meliponiculture sites in Riau Province, Indonesia. Values represent daytime measurements recorded during foraging hours at each site. Measurements were taken in a single dry-season window. Microclimate data were previously reported in a companion environmental study (Suhesti et al. 2025 manuscipt in review) and are reproduced here to provide ecological context for the nutritional dataset

Site/Village	Air temp (°C)	Relative humidity (%)	Wind speed (m s^{-1})	Light intensity (lux)	Soil pH
Kuok	32	76	0.0	44200	6.6
Umban Sari	34	66	0.6	42200	6.7
Usul	31	68	0.0	41000	5.6
Merempan Hilir	35	66	0.0	48500	5.2
Batu Rijal	35	63	0.9	48700	6.0

Table S2. Forage-vegetation diversity around stingless-bee meliponiculture sites in Riau Province, Indonesia. Vegetation diversity data were previously reported in a companion environmental study (Suhesti et al. 2025 manuscipt in review) and are reproduced here to provide ecological context for the nutritional dataset

Site/Village	Species richness	Plant individuals	Shannon-Wiener H′	Diversity level
Kuok	20	238	2.656	Moderate
Umban Sari	23	1683	2.615	Moderate
Usul	19	571	2.346	Moderate
Merempan Hilir	20	1362	1.629	Moderate
Batu Rijal	26	2161	2.697	Moderate

Table S3. Non-parametric effect size (Cliff's delta) for *H. itama* across locations Cliff's delta (δ) thresholds: \|δ\|≥0.474 (large)), 0.147-0.474 (medium), <0.147 (small). Positive δ indicates the first group tends to be larger

Parameter	Pair	Cliff's delta (δ)
Moisture content	Batu Rijal vs Kuok	1.0
Moisture content	Batu Rijal vs Siak	1.0
Moisture content	Batu Rijal vs Umban Sari	1.0
Moisture content	Batu Rijal vs Usul	1.0
Moisture content	Kuok vs Siak	1.0
Moisture content	Kuok vs Umban Sari	-0.333
Moisture content	Kuok vs Usul	-1.0
Moisture content	Siak vs Umban Sari	-1.0
Moisture content	Siak vs Usul	-1.0
Moisture content	Umban Sari vs Usul	-1.0
Ash	Batu Rijal vs Kuok	-1.0
Ash	Batu Rijal vs Siak	-1.0
Ash	Batu Rijal vs Umban Sari	1.0
Ash	Batu Rijal vs Usul	-1.0
Ash	Kuok vs Siak	1.0
Ash	Kuok vs Umban Sari	1.0
Ash	Kuok vs Usul	1.0
Ash	Siak vs Umban Sari	1.0
Ash	Siak vs Usul	-0.778
Ash	Umban Sari vs Usul	-1.0
Total energy	Batu Rijal vs Kuok	-1.0
Total energy	Batu Rijal vs Siak	-1.0
Total energy	Batu Rijal vs Umban Sari	-1.0
Total energy	Batu Rijal vs Usul	-1.0
Total energy	Kuok vs Siak	-0.778
Total energy	Kuok vs Umban Sari	-0.333
Total energy	Kuok vs Usul	-0.556
Total energy	Siak vs Umban Sari	0.333
Total energy	Siak vs Usul	0.556
Total energy	Umban Sari vs Usul	-0.111
Protein	Batu Rijal vs Kuok	-1.0
Protein	Batu Rijal vs Siak	-1.0
Protein	Batu Rijal vs Umban Sari	-1.0
Protein	Batu Rijal vs Usul	-1.0
Protein	Kuok vs Siak	1.0
Protein	Kuok vs Umban Sari	1.0
Protein	Kuok vs Usul	1.0
Protein	Siak vs Umban Sari	1.0
Protein	Siak vs Usul	1.0
Protein	Umban Sari vs Usul	-1.0
Carbohydrate	Batu Rijal vs Kuok	1.0
Carbohydrate	Batu Rijal vs Siak	1.0
Carbohydrate	Batu Rijal vs Umban Sari	-1.0
Carbohydrate	Batu Rijal vs Usul	0.111
Carbohydrate	Kuok vs Siak	-1.0
Carbohydrate	Kuok vs Umban Sari	-1.0
Carbohydrate	Kuok vs Usul	-1.0
Carbohydrate	Siak vs Umban Sari	-1.0
Carbohydrate	Siak vs Usul	-1.0
Carbohydrate	Umban Sari vs Usul	1.0
Total fat	Batu Rijal vs Kuok	-1.0
Total fat	Batu Rijal vs Siak	-1.0
Total fat	Batu Rijal vs Umban Sari	-1.0
Total fat	Batu Rijal vs Usul	-1.0
Total fat	Kuok vs Siak	1.0
Total fat	Kuok vs Umban Sari	1.0
Total fat	Kuok vs Usul	-1.0
Total fat	Siak vs Umban Sari	-1.0
Total fat	Siak vs Usul	-1.0
Total fat	Umban Sari vs Usul	-1.0
Dietary fibre	Batu Rijal vs Kuok	-1.0
Dietary fibre	Batu Rijal vs Siak	-1.0
Dietary fibre	Batu Rijal vs Umban Sari	-1.0
Dietary fibre	Batu Rijal vs Usul	-1.0
Dietary fibre	Kuok vs Siak	1.0
Dietary fibre	Kuok vs Umban Sari	1.0
Dietary fibre	Kuok vs Usul	-1.0

Dietary fibre	Siak vs Umban Sari	-1.0
Dietary fibre	Siak vs Usul	-1.0
Dietary fibre	Umban Sari vs Usul	-1.0
Vitamin A content	Batu Rijal vs Kuok	-1.0
Vitamin A content	Batu Rijal vs Siak	-1.0
Vitamin A content	Batu Rijal vs Umban Sari	-1.0
Vitamin A content	Batu Rijal vs Usul	-1.0
Vitamin A content	Kuok vs Siak	1.0
Vitamin A content	Kuok vs Umban Sari	1.0
Vitamin A content	Kuok vs Usul	-1.0
Vitamin A content	Siak vs Umban Sari	-1.0
Vitamin A content	Siak vs Usul	-1.0
Vitamin A content	Umban Sari vs Usul	-1.0
Vitamin B1 content	Batu Rijal vs Kuok	0.0
Vitamin B1 content	Batu Rijal vs Siak	0.0
Vitamin B1 content	Batu Rijal vs Umban Sari	0.0
Vitamin B1 content	Batu Rijal vs Usul	0.0
Vitamin B1 content	Kuok vs Siak	0.0
Vitamin B1 content	Kuok vs Umban Sari	0.0
Vitamin B1 content	Kuok vs Usul	0.0
Vitamin B1 content	Siak vs Umban Sari	0.0
Vitamin B1 content	Siak vs Usul	0.0
Vitamin B1 content	Umban Sari vs Usul	0.0
Vitamin B2 content	Batu Rijal vs Kuok	-1.0
Vitamin B2 content	Batu Rijal vs Siak	-1.0
Vitamin B2 content	Batu Rijal vs Umban Sari	1.0
Vitamin B2 content	Batu Rijal vs Usul	-1.0
Vitamin B2 content	Kuok vs Siak	1.0
Vitamin B2 content	Kuok vs Umban Sari	1.0
Vitamin B2 content	Kuok vs Usul	1.0
Vitamin B2 content	Siak vs Umban Sari	1.0
Vitamin B2 content	Siak vs Usul	0.111
Vitamin B2 content	Umban Sari vs Usul	-1.0
Vitamin B3 content	Batu Rijal vs Kuok	0.0
Vitamin B3 content	Batu Rijal vs Siak	0.0
Vitamin B3 content	Batu Rijal vs Umban Sari	0.0
Vitamin B3 content	Batu Rijal vs Usul	0.0
Vitamin B3 content	Kuok vs Siak	0.0
Vitamin B3 content	Kuok vs Umban Sari	0.0
Vitamin B3 content	Kuok vs Usul	0.0
Vitamin B3 content	Siak vs Umban Sari	0.0
Vitamin B3 content	Siak vs Usul	0.0
Vitamin B3 content	Umban Sari vs Usul	0.0
Vitamin B6 content	Batu Rijal vs Kuok	1.0
Vitamin B6 content	Batu Rijal vs Siak	1.0
Vitamin B6 content	Batu Rijal vs Umban Sari	1.0
Vitamin B6 content	Batu Rijal vs Usul	-1.0
Vitamin B6 content	Kuok vs Siak	1.0
Vitamin B6 content	Kuok vs Umban Sari	1.0
Vitamin B6 content	Kuok vs Usul	-1.0
Vitamin B6 content	Siak vs Umban Sari	-1.0
Vitamin B6 content	Siak vs Usul	-1.0
Vitamin B6 content	Umban Sari vs Usul	-1.0

Table S4. Typical/provisional validation and acceptance criteria for proximate and vitamin analyses of stingless bee bread. These criteria summarize typical/provisional validation windows for AOAC/SNI-compliant methods and were used as analytical acceptance thresholds in this study. Laboratory-specific validation sheets from the contracted ISO/IEC 17025 laboratory were not provided with the client report

Analyte / parameter	Matrix	Reference method / guidance	Internal instruction or code	Principle / instrument	Calibration range (typical)	Minimum accepted linearity (R^2)	Target recovery range (%)	Approximate LOD / LOQ (typical)
Moisture	Bee bread (lyophilised)	SNI 01-2891-1992, gravimetric moisture	Internal SOP: moisture (oven)	Oven drying to constant weight (gravimetric)	Not applicable	Not applicable	95-105	Not defined by calibration; detection limited by balance resolution (~0.1 mg)
Ash	Bee bread (lyophilised)	SNI 01-2891-1992, ash content	Internal SOP: ash (muffle furnace)	Muffle furnace at 550 °C (gravimetric)	Not applicable	Not applicable	95-105	Not defined by calibration; gravimetric end-point at constant mass
Crude protein (N×6.25)	Bee bread (lyophilised)	AOAC 2001.11; SNI 01-2891-1992 (protein by Kjeldahl)	Internal SOP: nitrogen and protein titrimetry	Kjeldahl digestion-distillation, titrimetric N determination	Not applicable	Not applicable	90-110	Sensitivity governed by titrant normality; LOQ not limiting at observed levels
Crude fat	Bee bread (lyophilised)	SNI 01-2891-1992, fat by Soxhlet	Internal SOP: crude fat (Soxhlet / hot extraction)	Soxhlet / hot extraction, gravimetric end-point	Not applicable	Not applicable	90-110	Not defined by calibration; gravimetric, limited by balance resolution
Carbohydrate (by difference)	Bee bread (lyophilised)	FAO (2003); BPOM (2019), carbohydrate by difference	Internal SOP: carbohydrate and energy by difference	Calculated from moisture, ash, protein, and fat	Not applicable	Not applicable	Depends on component methods	Not applicable; derived by difference
Vitamin B_2 (riboflavin)	Bee bread (lyophilised)	Water-soluble vitamins by UPLC (e.g. AOAC-based methods)	Internal UPLC SOP for B-group vitamins	UPLC, C18 column; gradient elution; PDA detection at ~265 nm	≈0.02-10 $\mu g \cdot mL^{-1}$ (standard mix)	≥0.995	80-120	LOD ≈0.01 $mg \cdot 100\ g^{-1}$; LOQ ≈0.03 $mg \cdot 100\ g^{-1}$
Vitamin A (retinol equivalents)	Bee bread (lyophilised)	AOAC 2001.13; HPLC determination of vitamin A	Internal HPLC SOP for fat-soluble vitamins	HPLC, C18 column; gradient methanol-water; PDA detection at ~325 nm	≈0.05-20 $\mu g \cdot mL^{-1}$ (retinol)	≥0.995	80-120	LOD ≈0.5 $\mu g \cdot 100\ g^{-1}$; LOQ ≈1.5 $\mu g \cdot 100\ g^{-1}$
Vitamin E (α-tocopherol)	Bee bread (lyophilised)	AOAC 2001.13; HPLC determination of vitamin E	Internal HPLC SOP for fat-soluble vitamins	HPLC, C18 column; gradient methanol-water; PDA detection at ~290 nm	≈0.05-20 $\mu g \cdot mL^{-1}$ (α-tocopherol)	≥0.995	80-120	LOD ≈0.5 $\mu g \cdot 100\ g^{-1}$; LOQ ≈1.5 $\mu g \cdot 100\ g^{-1}$

Table S5. Dataset-specific QA/QC metrics for proximate and vitamin analyses of stingless bee bee bread (technical triplicates and proximate mass-balance). Technical precision is summarized as the median and range of relative standard deviations (%RSD) across seven bee-bread composite samples, each analyzed in triplicate. Proximate mass-balance is calculated as the sum of moisture, ash, crude protein, crude fat, and carbohydrate on a percentage basis

Analyte / parameter	Number of composite samples (each n = 3 technical replicates)	Median %RSD across samples	Range of %RSD (min-max)	Acceptance criterion (%RSD)	Comment on performance
Moisture	7	0.53	0.17-0.70	≤5	Well within acceptance limits
Ash	7	1.32	0.23-1.59	≤5	Well within acceptance limits
Crude protein	7	0.82	0.37-1.28	≤5	Well within acceptance limits
Crude fat	7	1.63	1.12-1.90	≤5	Well within acceptance limits
Carbohydrate (by difference)	7	0.43	0.08-0.73	≤5	Well within acceptance limits
Vitamin A	6	0.25	0.08-1.33	≤10	Well within acceptance; far from LOQ
Vitamin B_2	6	1.00	0.00-1.70	≤10	Within acceptance; some triplicates identical by rounding
Vitamin E	7	0.64	0.26-1.53	≤10	Well within acceptance limits

Additional QA metric: Proximate mass-balance closure

QA metric	Definition	Median value across samples	Range (min-max)	Acceptance criterion	Comment
Proximate mass-balance	Sum of moisture, ash, crude protein, crude fat, and carbohydrate (% w/w)	100.00%	100.00-100.06%	90-110% (nutritional laboratories)	Indicates excellent closure and internal consistency