

Exploration of intestinal microbiota of breastfeeding mothers with the habit of drinking dangke water as a local food of the Enrekang community

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ABSTRACT

Background: Dangke water is habitually consumed by breastfeeding mothers in Curio District, Enrekang Regency, yet evidence describing their gut microbiota profile remains limited.

Objective: Describe gut microbiota profile of breastfeeding mothers who have a habit of consuming dangke water.

Method: Descriptive cross-sectional study involving 42 breastfeeding mothers, consisting of 33 mothers from Curio District who habitually consumed dangke water and 9 mothers from Kabere District who did not. Field visits were conducted only to document habitual intake of ~150 mL/day over three months and not as an intervention, acknowledging the small and unbalanced sample size (33 vs. 9). Data collected included individual characteristics, nutritional intake, and fecal samples. Fecal samples were analyzed using 16S rRNA gene sequencing at the HUM-RC Unhas Laboratory, followed by descriptive bioinformatic analysis.

Results: A total of 42 breastfeeding mothers participated, mostly aged 30–39 years (45.2%), with high school education (47.6%) and working as housewives (54.8%). All findings were described descriptively without inferential statistical testing. Dangke water consumption group showed higher species richness than the non-consumption group (77.00 ± 17.95 vs 55.73 ± 16.51). Bacillota predominated in

both groups, while the consumption group showed a higher proportion of Bacteroidota. Higher relative abundances of *Faecalibacterium prausnitzii* (13.32% vs 12.37%), *Agathobacter rectalis* (7.14% vs 5.82%), *Blautia wexlerae* (5.54% vs 4.60%), and *Segatella copri* (5.60% vs 4.81%) were observed based solely on descriptive metagenomic profiling.

Conclusion: Dangke water consumption was descriptively accompanied by higher microbial richness and a greater relative abundance of butyrate-producing bacteria in breastfeeding mothers.

KEYWORDS

Bacterial diversity; butyrate-producing bacteria; traditional foods; metagenomics; maternal nutrition.

INTRODUCTION

Nutritional intake of breastfeeding mothers in Indonesia is still low, with energy and macronutrient adequacy <80% of the RDA, while micronutrients such as vitamin D (<70% EAR), vitamin E (<50% EAR), vitamin B complex (<80% EAR), and minerals such as calcium, potassium, and iron generally do not meet 60–80% of the requirement¹⁻³. This finding is in line with several studies in various Community Health Centers in Makassar City which show that energy intake of breastfeeding mothers is still in the range of 53–86% of the RDA, protein 21–94% of the RDA, and many carbohydrates and fats also do not reach adequacy <80% of the RDA. This condition indicates that nationally and locally, breastfeeding mothers still experience nutritional deficits that can affect the quality and quantity of breast milk⁴⁻⁸.

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In Indonesia, there are several regional foods and drinks consumed by breastfeeding mothers as part of local traditions, one of which is a local cheese made from cow's or buffalo's milk known as "Dangke." The liquid waste from the dangke-making process is highly sought after by various groups, including breastfeeding mothers, especially in the Curio District of Enrekang Regency. Traditionally, this waste is called "air dangke" and is believed to help mothers recover after childbirth and increase breast milk production.

Dangke water, a byproduct of the production of Enrekang's signature dangke, is rich in whey protein, and our initial research indicates that the waste contains 12.5 ng/ml of aLa. This component is an important part of the HAMLET (Human Alpha-Lactalbumin Made Lethal to Tumor cells) complex, which is known to have anti-cancer and immunomodulatory activity and has the potential to improve breast milk quality while influencing the gut microbiota of breastfeeding mothers^{9,10}. The gut microbiota is a complex ecosystem with more than 100 trillion cells that plays a role in cross-organ homeostasis, metabolism, immunity, and the production of short-chain fatty acids¹¹⁻¹³. Microbiota imbalance (dysbiosis) can trigger metabolic syndrome and degenerative diseases¹⁴. Its composition is influenced by diet, including the type of protein^{15,16}. Consuming whey protein such as dangke water is believed to support the growth of probiotic bacteria and suppress pathogens, even the natural LAB in it can directly act as a probiotic¹⁷.

ALA and OA levels in breast milk in Indonesia are still lower than those in Asian, New Zealand, and Maori ethnicities^{18,19}. An intervention of 20 ml/day of Extra Virgin Olive Oil (EVOO) for 14 days increased OA levels in breast milk without affecting maternal body composition²⁰⁻²². However, limited access to EVOO emphasizes the need for local food-based alternatives. The bioactive potential of dangke water opens up opportunities for the development of local functional foods that are beneficial for breastfeeding mothers.

Low nutritional intake in breastfeeding mothers indicates the need for education and Supplementary Feeding (PMT) specifically for breastfeeding mothers. Currently, the national program only provides education in the form of balanced nutrition leaflets for breastfeeding mothers, but there is no PMT specifically formulated for this group. To develop PMT for breastfeeding mothers, local foods or drinks such as dangke water can be utilized, either in their natural form or modified and enriched with additional nutrients so that they become more practical, attractive, and can fill nutritional deficiencies that have not been met. Improving the intake of breastfeeding mothers is believed to increase the volume and content of breast milk, which in turn can improve the national exclusive breastfeeding coverage which is currently around 66%, still below the national target of 80% and the WHO global target of 70%¹.

METHOD

This study is a descriptive cross-sectional study that aims to identify the gut microbiota profile in breastfeeding mothers who habitually consume dangke water. A total of 42 breastfeeding mothers were included, consisting of 33 mothers who reported regular consumption of dangke water from Curio District (sample numbers 1–33) and 9 mothers who reported no consumption of dangke water from Kabere District (sample numbers 34–42). These groups were defined based on self-reported habitual consumption patterns and were not designed as intervention or experimental groups. The unequal and limited sample size between groups (33 vs. 9) is acknowledged as a methodological limitation that restricts comparability and internal validity. Field visits to the homes of mothers in the dangke water consumption group were conducted solely to document and confirm habitual intake of approximately 150 mL/day, and not as an intervention, treatment, or longitudinal follow-up. No causal or associative effects were evaluated in this study.

Data collected included individual characteristics, nutritional intake using a 24-hour dietary recall and a semi-quantitative food frequency questionnaire (SQ-FFQ), and nutritional status through anthropometric measurements. Inclusion criteria were breastfeeding mothers aged 18–45 years who had been breastfeeding for at least one month. Exclusion criteria included the use of antibiotics, probiotics, or prebiotics within the last three months; presence of chronic or gastrointestinal diseases; acute infections at the time of sampling; and refusal to provide informed consent. These criteria were applied to minimize potential confounding factors affecting gut microbiota composition.

All stool samples were collected using DNA stabilizer tubes and stored frozen until ready for analysis. Laboratory analysis was conducted at the HUM-RC Laboratory of Hasanuddin University. Procedures included microbial DNA extraction followed by 16S rRNA gene sequencing using the Oxford Nanopore Technologies platform.

Bioinformatic analysis was performed using a wf-metagenomics sequencing pipeline to obtain taxonomic profiles. Alpha diversity indices (including species richness) were calculated descriptively. Beta diversity analysis and inferential statistical comparisons between groups were not performed, and no level of statistical significance was applied, in accordance with the descriptive nature of the study.

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Health Research Ethics Committee of Hasanuddin University, Indonesia (Approval No: **00182/UN4.1.20/KEP/2025**). Written informed consent was obtained from all participants prior to data and sample collection, and confidentiality of participant information was strictly maintained throughout the study.

RESULTS

A total of 42 breastfeeding mothers participated in this study. Table 1 presents the characteristics of the dangke water-consuming group only (N = 33), while characteristics of the non-consumption group (N = 9) were not analyzed separately due to the limited sample size. This distinction is made to avoid misinterpretation of baseline comparability between groups.

The majority of respondents were aged 30–39 years (45.2%), followed by 20–29 years (42.9%). Respondents aged <20 years accounted for 4.8%, and those aged ≥40 years accounted for 7.1%. Most mothers had a high school education (47.6%), followed by elementary–junior high school (33.3%), while 19.0% had a diploma or bachelor's degree. More than half of respondents were housewives (54.8%), followed by farmers (26.2%), civil servants (11.9%), and traders (7.1%). The largest proportion of breastfed children was aged 3–4 months (38.1%), followed by 5–6 months (35.7%) (Table 1).

Alpha diversity indices are presented descriptively without inferential statistical testing. No p-values were calculated, and differences between groups should be interpreted as observed patterns only. The dangke water consumption group (n = 33) showed a higher observed richness (77.00 ± 17.95)

compared to the non-consumption group (55.73 ± 16.51), indicating a higher number of observed bacterial taxa. Shannon and Simpson indices were relatively similar between groups, suggesting comparable microbial diversity and evenness. The control group showed slightly higher Pielou's evenness (0.75 ± 0.07 vs. 0.72 ± 0.06).

The effective number of species was descriptively higher in the consumption group (24.09 ± 8.86 vs. 20.88 ± 6.13). Total read counts were also higher in the consumption group; however, these values reflect sequencing output and should not be interpreted as biological abundance or statistical differences. Given the descriptive study design and the small size of the control group, no inferential statistical analysis was performed, and all findings should be interpreted with caution. Overall, alpha diversity results indicate descriptive differences between groups without evidence of statistical significance (Table 2).

At the phylum level, Bacillota was dominant in both groups (82.86% in the consumption group vs. 85.28% in the non-consumption group). The proportion of Bacteroidota was descriptively higher in the dangke water consumption group (7.21% vs. 6.27%), although the absolute difference was small and not statistically tested.

The phylum previously labeled as "Pseudomonads" has been corrected to "Pseudomonadota" (9.48% vs. 8.18%). Other phyla were present in very low proportions (<0.3%). No causal or functional interpretation is inferred from these

Table 1. Characteristics of Breastfeeding Mothers (N = 42)

Variables	Category	n	%
Mother's age (years)	<20	2	4.8
	20–29	18	42.9
	30–39	19	45.2
	≥40	3	7.1
Education	Elementary–Middle School	14	33.3
	Senior High School	20	47.6
	D3/S1	8	19.0
Work	Housewife	23	54.8
	Farmer	11	26.2
	Trader	3	7.1
	civil servant	5	11.9
Child's age (months)	1–2	8	19.0
	3–4	16	38.1
	5–6	15	35.7
	7–8	3	7.1

Table 2. Alpha Diversity Indices of Gut Microbiota (Shannon, Simpson, Chao1)

Index	Mean ± SD Intervention (n=33)	Mean ± SD Control (n=9)
Richness	77.00 ± 17.95	55.73 ± 16.51
Shannon diversity index	3.11 ± 0.37	2.99 ± 0.31
Simpson's index	0.91 ± 0.05	0.90 ± 0.05
Inverse Simpson index	1.11 ± 0.06	1.11 ± 0.07
Pielou's evenness	0.72 ± 0.06	0.75 ± 0.07
Fisher's alpha	38.24 ± 1.21	45.13 ± 4.34
Berger–Parker index	0.21 ± 0.10	0.22 ± 0.09
Effective number of species	24.09 ± 8.86	20.88 ± 6.13
Total counts	22,409.56 ± 3,708.36	11,540.79 ± 6,179.11

compositional differences due to the descriptive nature of the analysis (Table 3, Figure 1).

At the species level, the relative abundances of several taxa were descriptively higher in the dangke water consumption group, including *Faecalibacterium prausnitzii*

(13.32% vs. 12.37%), *Agathobacter rectalis* (7.14% vs. 5.82%), *Blautia wexlerae* (5.54% vs. 4.60%), and *Segatella copri* (5.60% vs. 4.81%). Conversely, some species such as *Blautia massiliensis* (7.47% vs. 3.95%) and *Dorea longicatena* (5.40% vs. 2.44%) showed higher relative abundance in the

Table 3. Average Relative Abundance of the Top 10 Phyla and Their Dominant Species in Metagenomic Samples

Phylum	Average (%) Intervention (n=33)	Average (%) Control (n=9)	Species	Average (%) Intervention (n=33)	Average (%) Control (n=9)
Bacillota	82.86	85.28	<i>Faecalibacterium prausnitzii</i>	13.32	12.37
Bacteroidota	7.21	6.27	<i>Agathobacter rectalis</i>	7.14	5.82
Pseudomonadota	9.48	8.18	<i>Blautia wexlerae</i>	5.54	4.60
Other	0.33	0.28	<i>Segatella copri</i>	5.60	4.81
Thermodesulfobacteriota	0.09	0.02	<i>Blautia massiliensis</i>	3.95	7.47
Cyanobacteriota	0.04	0.00	<i>Succinivibrio dextrinosolvens</i>	4.72	5.47
Actinomycetota	0.02	0.03	<i>Blautia obeum</i>	3.21	3.63
Fusobacteriota	0.03	0.00	<i>Coprococcus eutactus</i>	2.57	3.34
Lentisphaerota	0.02	0.01	<i>Dorea longicatena</i>	2.44	5.40
Rhodothermota	0.01	0.00	<i>Anaerobutyricum hallii</i>	2.84	3.75

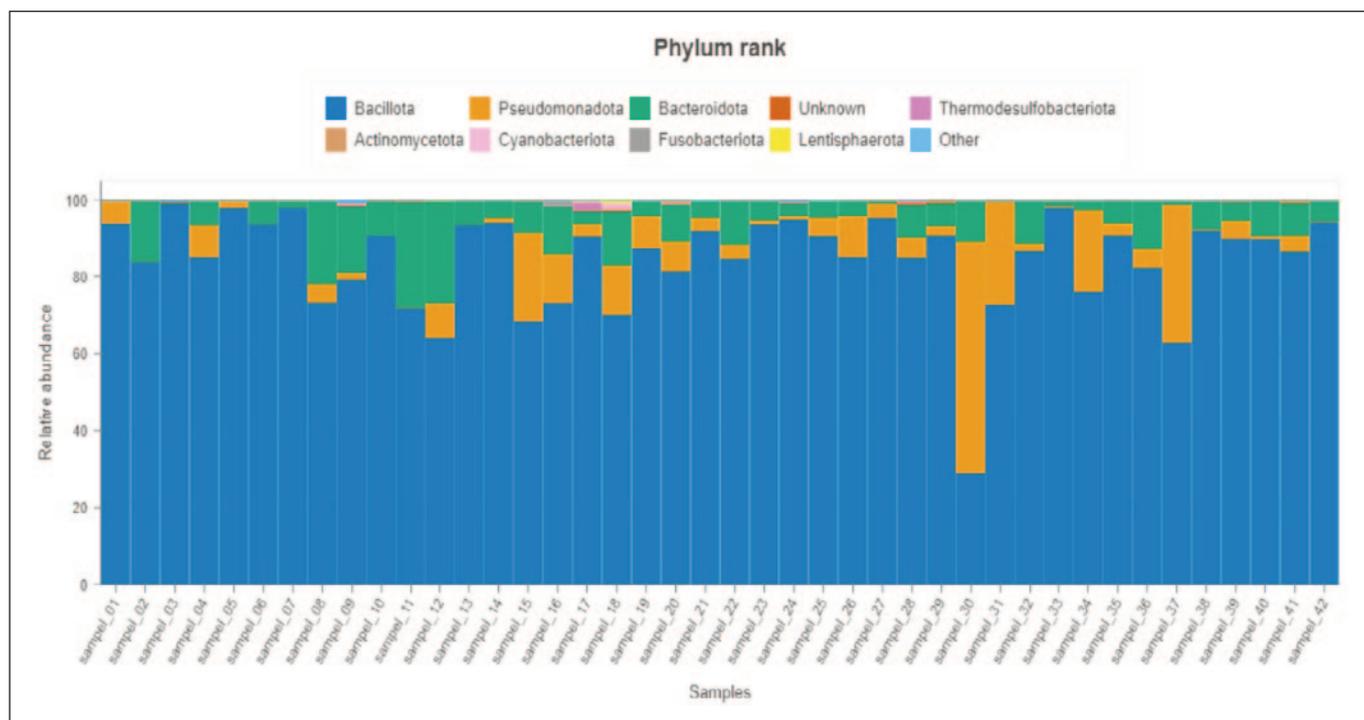


Figure 1. Relative Abundance of Gut Microbiota at the Phylum Level Across Samples

non-consumption group. These findings are based solely on descriptive metagenomic profiling, and no statistical testing was performed to assess significance or clinical relevance (Table 3, Figure 2).

DISCUSSION

This study is an initial attempt to explore the potential of dangke water as a local food-based nutritional intervention in influencing the gut microbiota profile in breastfeeding mothers. The results showed that the gut microbiota profile in both groups was dominated by the phylum Bacillota, namely 82.86% in the intervention group and 85.28% in the control group. This dominance is in line with the microbiota pattern in populations consuming fermented foods, where dairy or fermented foods play a role in supporting the growth of the Bacillota bacterial group, including butyrate-producing bacteria such as *Faecalibacterium prausnitzii*²³. In the context of local food culture, dangke—a traditional dairy product processed through natural coagulation—contains bioactive peptides, lactic acid, and other fermentative components that have the potential to support the colonization of beneficial bacteria.

The main difference between the two groups was seen in the alpha-diversity values. The intervention group showed higher richness (77.00 ± 17.95) compared to the control group (55.73 ± 16.51). Although the Shannon and Simpson indices between the two groups were relatively similar, the higher richness and effective species values in the intervention group indicated a richer and more diverse microbiota

community. These findings are in line with evidence showing that fermented food consumption can increase microbiota diversity through direct and indirect exposure to fermentative metabolites²⁴. Sonnenburg and Sonnenburg (2019) also reported that regular consumption of fermented foods increased microbiota diversity and reduced the dominance of certain taxa²⁵. Thus, the consumption of dangke water as part of a traditional diet likely contributed to the high richness and total microbial counts in the intervention group.

At the species level, the intervention group showed a higher proportion of short-chain fatty acid (SCFA)-producing bacteria, such as *F. prausnitzii* (13.32% vs. 12.37%), *Agathobacter rectalis* (7.14% vs. 5.82%), *Blautia wexlerae* (5.54% vs. 4.60%), and *Segatella copri* (5.60% vs. 4.81%). SCFAs play an important role in maintaining intestinal epithelial integrity, reducing inflammation, and strengthening the mucosal immune system²⁶. Fermented milk products such as dangke can provide fermentable substrates that support the growth of these SCFA-producing bacteria. Scientific studies have shown that a diet high in fermented foods can increase the abundance of butyrate bacteria while improving the composition of the gut microbiota²⁷. This strengthens the finding that breastfeeding mothers who consume dangke water have a more favorable microbiota profile than the control group.

The high dominance of Bacillota in both groups is consistent with the microbiota patterns of people with a diet high in fermentation and fiber. Individuals who rarely consume fermented foods generally experience a decrease in Bacillota dominance and an increase in Bacteroidota or Pseudomona-

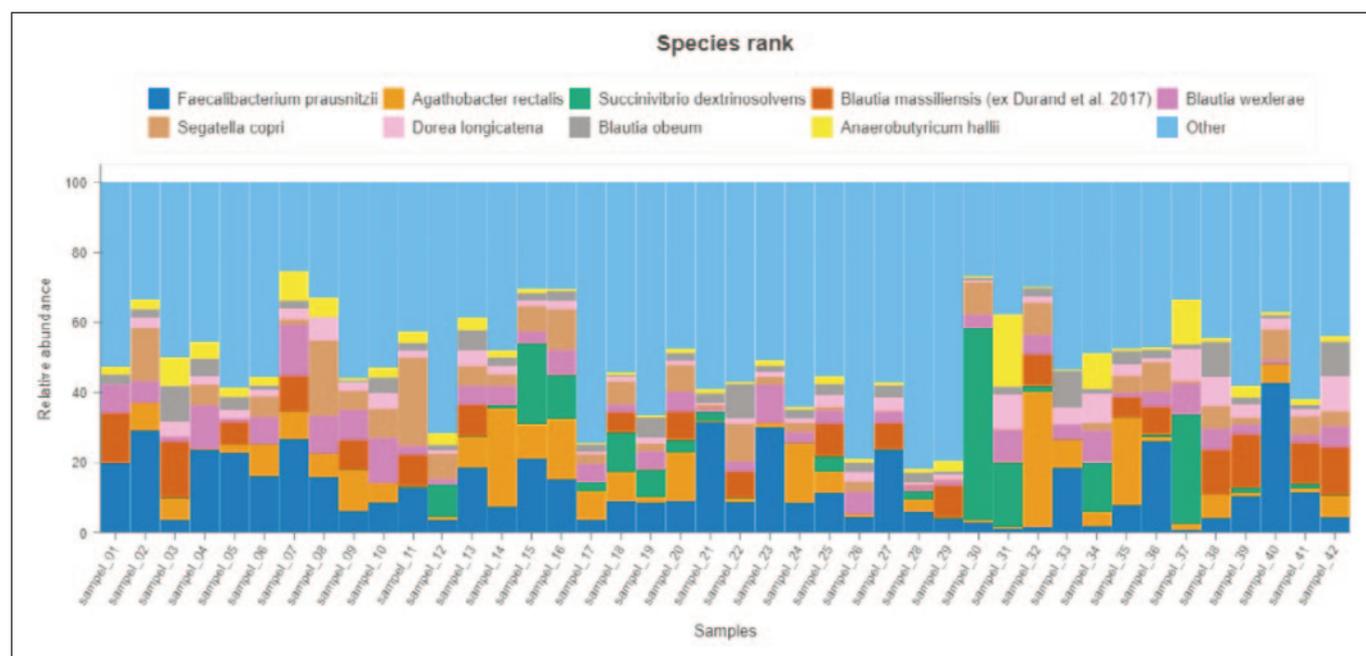


Figure 2. Relative abundance of dominant bacterial species detected in all samples based on metagenomic sequencing

dota²⁸. In this study, the intervention group showed an increase in Bacteroidota (7.21% vs. 6.27%), indicating better complex carbohydrate fermentation activity. This strengthens the possibility that dangke water consumption provides a protective effect by stimulating fermentative bacteria and maintaining the stability of the microbiota community.

The presence of species such as *Segatella copri* and *Sucinivibrio dextrinosolvens*, which remained present in both groups, supports the theory that traditional fermented food-based diets can create a relatively homogeneous microbiota profile. Previous studies have shown that populations with traditional diets have a distinctive microbiota fingerprint that differs from modern populations with a high intake of ultra-processed foods²⁹.

Variations in composition between individuals were still found, but the tendency for higher fermentative bacteria in the intervention group suggests that consuming dangke water can strengthen the stability of the microbial community. In breastfeeding mothers, this is important because a balanced microbiota composition can influence metabolic health, immunity, and the bioactive composition of breast milk. SCFAs produced by dominant bacteria play a role in modulating maternal immunity and may even contribute to the development of the infant's immune system through breast milk³⁰.

CONCLUSION

The gut microbiota profile of breastfeeding mothers in this study—Bacillota dominance, high abundance of *F. prausnitzii*, and good community diversity—is consistent with a healthy gut community and potentially supports gastrointestinal health. Consumption patterns of traditional dairy-based local foods (such as dangke) likely contribute to this profile, but a causal relationship needs to be established through further studies. These findings open important avenues of research into the interactions between traditional diets, gut microbiota, and maternal-infant health in local populations.

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