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Impact of Early Food Diversification on the Composition of the Gut Microbiota in Infants during the First 200 Days of Life from Three Municipalities in the District of Abidjan

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Abstract

The gastrointestinal microbiota plays a crucial role in host health by modulating nutrition and disease processes. Although gut microbial composition is known to vary with diet, few studies have explored how specific infant diets influence the gut microbiota. This study compares the fecal microbiota profiles of three groups of infants: those exclusively breastfed with complementary food (BF + FD), formula-fed with complementary food (FF + FD), and infants receiving a combination of breast milk, formula, and complementary food (BF + FF + FD). Fecal microbiota was analyzed through Illumina high-throughput sequencing. The results reveal that Actinobacteria and Bacteroides are the dominant phyla across all groups. However, Firmicutes and Bacteroidetes are underrepresented in infants fed with FF + FD. Among infants on the BF + FD diet, Bifidobacterium (17.84%), Escherichia-Shigella (9.37%), and Streptococcus (7.4%) were the most prevalent. In the FF + FD group, Bifidobacterium (24.33%) and Escherichia-Shigella (14.35%) dominated. The BF + FF + FD group showed similar trends, with Bifidobacterium (14.99%), Escherichia-Shigella (9.17%), and Streptococcus (7.68%) prevailing ($p = 0.05$). Age also influenced microbial composition. Between 0 - 119 days, Bifidobacterium (15.70%) and Enterobacteriaceae (11.28%) predominated, while at 120

- 179 days, these proportions shifted to 20.62% and 8.95%, respectively. By 200 days, Bifidobacterium and Enterobacteriaceae were still present but in lower proportions (14% and 9.31%).

Keywords

Infant Gut Microbiota, Breastfeeding, Formula Feeding, Complementary Food, Illumina Sequencing

1. Introduction

The infant gut microbiota undergoes significant changes during the first years of life, both in terms of taxonomic composition and diversity. As the infant is exposed to new foods and environments, the microbial diversity within the gut increases (referred to as alpha diversity). The establishment and development of this intestinal flora are complex, influenced by various factors such as mode of delivery, feeding practices, and environmental bacterial exposure. At birth, the intestinal tract is sterile, and initial colonization by maternal vaginal or dermal flora begins within hours, achieving an initial microbial balance within 1 to 2 weeks.

The composition of the infant gut microbiota is highly susceptible to modulation by external factors, including delivery mode (vaginal vs. Cesarean), antibiotic exposure, genetics, home environment, geographic location, and whether the child is raised in an urban or rural setting. Feeding practices also play a crucial role, with breast milk, formula, or mixed feeding patterns influencing microbial composition. While the infant microbiota differs significantly from that of adults, it tends to converge toward an adult-like microbial community between 2 to 3 years of age [1]-[3].

Among these influencing factors, diet stands out as a key determinant in shaping the gut microbiota during early life [4] [5]. Numerous studies in Europe [6] [7] and Asia [8] have examined how breastfeeding, formula feeding, and early dietary diversification affect the microbial composition of infants. However, there is limited research on how early dietary patterns impact the gut microbiota in developing countries, including Côte d'Ivoire.

Given the profound influence of diet on microbial composition and its implications for health [9], it is essential to better understand the effects of dietary practices on the gut microbiota during infancy. This period is particularly critical, as the first 200 days of life coincide with the rapid development and stabilization of the intestinal flora. The objective of this study is to assess the impact of the early introduction of foods on the gut microbiota of infants in three districts of Abidjan, Côte d'Ivoire.

2. Materials and Methods

2.1. Infant Enrollment

We enrolled 26 healthy infants from three communes in Abidjan, Côte d'Ivoire:

Abobo, Cocody, and Marcory (Table 1). The infants were divided into six groups: **BF**: Breastfed only; **BF + FD**: Breastfed + Food Diversification; **FF**: Formula-fed only; **FF + FD**: Formula-fed + Food Diversification; **BF + FF**: Breastfed + Formula-fed; **BF + FF + FD**: Breastfed + Formula-fed + Food Diversification. All infants were 0 - 200 days of age; exclusive breast-feeding, formula-feeding, mixed-feeding and infants received additional foods were established throughout the research period. In addition to the feeding pattern, there were no significant differences in gestational age and there was no use of antibiotics for infants in the six groups. The infants did not have any gastrointestinal diseases. Infants were not born by caesarean section. We grouped the infant based on the different diet categories identified during the surveys. The infants were divided into six feeding groups based on their feeding practices:

1) BF: Breastfed only

2) BF + FD: Breastfed + Food Diversification

3) FF: Formula-fed only

4) FF + FD: Formula-fed + Food Diversification

5) BF + FF: Breastfed + Formula-fed

6) BF + FF + FD: Breastfed + Formula-fed + Food Diversification

The infants were also categorized into three age groups:

- **0 - 119 days**
- **120 - 179 days**
- **180 - 200 days**

Table 1 provides an overview of the characteristics of the studied samples.

Table 1. Characteristics of the studied samples.

Sample Name	Commune	Age	Sex	Feeding Practice
Infant 1	Cocody	119 days	M	BF + FD
Infant 2	Cocody	120 days	F	BF + FD
Infant 3	Marcory	119 days	F	BF + FF + FD
Infant 4	Abobo	119 days	M	BF + FD
Infant 5	Marcory	60 days	F	BF + FD
Infant 6	Abobo	119 days	M	BF + FF + FD
Infant 7	Abobo	180 days	M	BF + FF + FD
Infant 8	Abobo	180 days	F	BF + FF + FD
Infant 9	Abobo	180 days	M	BF + FD
Infant 10	Abobo	30 days	F	BF + FD
Infant 11	Abobo	180 days	F	FF + FD
Infant 12	Marcory	180 days	F	BF + FD
Infant 13	Abobo	30 days	F	BF + FD
Infant 14	Abobo	180 days	M	BF + FF + FD
Infant 15	Abobo	150 days	M	BF + FF + DA
Infant 16	Cocody	120 days	M	BF + FF + DA
Infant 17	Abobo	90 days	M	BF + FF + DA
Infant 18	Marcory	120 days	F	BF + FF + DA
Infant 19	Abobo	180 days	F	BF + FD
Infant 20	Marcory	180 days	F	BF + FF + DA

Continued

Infant 21	Abobo	90 days	F	BF + FF + DA
Infant 22	Marcory	90 days	F	BF + FF + DA
Infant 23	Abobo	120 days	F	BF + FD
Infant 24	Marcory	150 days	M	BF + FD
Infant 25	Marcory	120 days	M	FF + FD
Infant 26	Abobo	150 days	M	BF + FD

Legend: M: Male; F: Female.

2.2. Faecal Sample

Collection and DNA Extraction

In all populations, fecal samples were collected from each individual in the morning and kept at −80˚C until extraction. DNA was extracted according to the protocol described by the manufacturer of the QIAamp PowerFecal Pro DNA KIT. The extracted DNA was stored at −20˚C until processed. To access the faecal microbiota, the V3 - V4 hypervariable region of the 16S rRNA gene was amplified using the primers (V3 - V4): 341F->CCTACGGGNGGCWGCAG 805R->GACTACHVGGGTATCTAATCC [10]. The PCR was done in two (2) steps, with Illumina Nextera adapters. The conditions of the first PCR: Enzyme Phusion (Thermofisher) Concentration of the primers: 0.4 µM final. The first PCR amplification was carried by 33 cycles at 55˚C for 30 s. The use of positive (+) and negative (−) controls for the PCR was carried out and the PCR products were visualized on an agarose gel. The amplicons are diluted to 1/50 and used as a template for the 2nd PCR (also called barcoding or indexing since this step is used to attach the indexes and the Illumina adapters necessary for Miseq sequencing).

2.3. Visualization of PCR Products on Agarose Gel

Amplicons were pooled (5 µL each) to generate the library. The pooled amplicons were subsequently purified using Ampure beads (Beckman) at a 0.7 ratio. Quality control of the library was conducted using Qubit HS dsDNA (Invitrogen) and quantitative PCR (qPCR) with the NEBNext® Library Quant Kit for Illumina (New England Biolabs). The library profile was analyzed on a Bioanalyzer using a DNA HS chip (Agilent Technologies). The library was sequenced on a MiSeq (Illumina) using a MiSeq V3 kit, PE300, with a 12% Phix spike-in. The sequencing reads were processed by the MiSeq, and the resulting FASTQ files were analyzed using the bioinformatics platform at CERMOFC (Montreal, Quebec, Canada).

2.4. Data Processing and Analysis

Raw sequences from all analyzed samples were demultiplexed, and adapters and barcodes were removed. The quality of the sequences was then assessed using FastQC, following a previously established protocol [11] [12]. The resulting data files were imported and processed within the R environment using various scripts and built-in functions available in different R packages. The sequences were subsequently trimmed using Trimmomatic [13]. Quality control post-trimming was

performed with FastQC [11] and MultiQC [12]. Finally, the filtered and trimmed files were processed using the "filterAndTrim" function from the DADA2 package [14].

Low-quality bases were discarded prior to analysis. The DADA2 (Divisive Amplicon Denoising Algorithm) pipeline was employed to process the filtered data files. Steps including dereplication, core denoising algorithm application, and base pair fusion were performed to obtain fully denoised sequences. The "removeBimeraDenovo" function from the DADA2 package was then utilized to eliminate chimeric sequences. Data cleaning was conducted through decontamination, denoising, and rarefaction, employing the decontam [15] [16] and vegan [17] packages. Amplicon sequence variant (ASV) sequences were assigned taxonomy using the Silva database (Silva_nr_v132) [18] with the "assignTaxonomy" function from the DADA2 package, and a phyloseq data object was created. A phylogenetic tree for taxa was constructed using the R package **ape**, leveraging the prune_taxa functions from the **phyloseq** package [15]. Differential analyses were conducted to identify differentially abundant taxa for each parameter using **DESeq2** [19].

2.5. Structure and Diversity of the Intestinal Microbiota across Different Diets

Illumina MiSeq high-throughput sequencing of the V3 - V4 hypervariable regions of the 16S rRNA gene was conducted to characterize the bacterial lineages present in the fecal microbiota of 26 infants. A total of 1,564,001 filtered taxonomic 16S rRNA gene sequences were generated, yielding a mean (±SD) of 62.55 ± 8.35 sequences per sample. The Chao1, ACE, Simpson, and Shannon diversity indices were employed to assess species richness and evenness of amplicon sequence variant (ASV) distribution within the bacterial community. The richness and diversity indices of the observed bacterial community are reported. The ACE index ranged from 174 to 580, while Chao1 richness varied between 174 and 590.

The Shannon and Simpson indices ranged from 1.49 to 4.55 and from 2.77 to 60.35, respectively. Notably, the fecal samples, particularly sample A24, exhibited the highest values compared to the others. The analysis of alpha diversity, as indicated by the Shannon species richness, revealed a significant microbial diversity in relation to dietary types (Kruskal-Wallis test, $p = 0.05$) and the age of the infants (Kruskal-Wallis test, $p < 0.05$). The majority of tag sequences had a length of approximately 50 bp, which aligns with the expected length of most bacterial V3 - V4 regions.

2.6. Ethical Considerations

This study was approved by the Ethics Committee of Côte d'Ivoire. Informed consent was obtained from the mothers of all enrolled infants.

2.7. Statistical Analyses

Differences between populations were analyzed using both parametric (ANOVA)

and nonparametric statistical methods. All results are presented as mean values (± SE), and differences between groups were considered significant at $p < 0.05$.

3. Results and Discussion

In this study, we characterized the fecal microbiota of 26 healthy infants from three districts: Abobo, Cocody, and Marcory. The children were selected based on their dietary habits, encompassing three distinct food typologies constituting the infants' diets. Food diversification included porridge made from millet, rice, maize, infant cereal, as well as anagobaka, honey, and orange juice.

The study you describe focuses on characterizing the bacterial lineages present in the intestinal microbiota of infants using 16S rRNA gene sequencing. Here's a breakdown of the main points:

Sequencing Method: Pyrosequencing of the V3 - V4 hypervariable regions of the 16S rRNA gene was conducted using Illumina MiSeq technology. This high-throughput sequencing generated 1,564,001 filtered sequences, with a mean of 62.55 ± 8.35 sequences per sample.

Alpha Diversity: The alpha diversity indices used in this analysis include:

- **ACE (Abundance-based Coverage Estimator)**: Values range from 174 to 580.
- **Chao1**: Values range from 174 to 590.
- **Shannon Index**: Varies between 1.49 and 4.55.
- **Simpson Index**: Varies between 2.77 and 60.35.

Infant 7 exhibits the highest alpha diversity, based on all the indices, suggesting a higher richness and evenness of bacterial species in their fecal sample.

Influence of Diet and Age:

- **There** is a significant difference in microbial diversity depending on the type of diet (Kruskal-Wallis test, $p = 0.05$).
- **Age** also influences microbial diversity (Kruskal-Wallis test, $p < 0.05$).

Primer Information: The primers used for this sequencing are around 50 bp in length, which is consistent with the size of most bacterial V3 - V4 regions.

This type of study is essential for understanding how different feeding practices (such as breastfeeding, formula feeding, or mixed diets) and age influence the development of the gut microbiota in infants. The data suggest that diet plays a key role in shaping microbial diversity, which could have long-term implications for infant health.

3.1. Microbial Richness and Diversity

To estimate microbial richness based on observed Amplicon Sequence Variants, the Chao1 index was used, while biodiversity was assessed using the Shannon index across the three groups. Significance was determined at a threshold of 0.05 (**Table 2**). No significant differences in richness or biodiversity were observed between the BF + FD, FF + DA, and BF + FF + FD samples at this threshold.

Table 2. Richness and diversity indices for each fecal sample.

Sample Name	Read	ASV	Ace	Chao1	Shannon	Simpson
Infant 1	74.9	39	282	271	2.28	4.94
Infant 2	72	39	378	408	2.08	3.62
Infant 3	67	40	466	473	2.35	3.90
Infant 4	65.6	39	559	563	3.23	7.84
Infant 5	70.1	39	359	361	2.18	3.64
Infant 6	42.2	39	392	470	4.55	60.35
Infant 7	56.8	39	580	590	3.63	13.41
Infant 8	67.2	39	243	230	3.17	13.74
Infant 9	75.5	39	431	433	2.19	4.25
Infant 10	52.7	39	248	236	3.15	13.12
Infant 11	71.1	40	220	207	2.53	5.34
Infant 12	62.2	39	326	324	2.73	7.49
Infant 13	52.8	40	494	511	3.96	22.90
Infant 14	62.9	39	299	307	2.57	5.34
Infant 15	64.7	39	293	292	2.51	6.01
Infant 16	54.8	39	475	467	3.49	13.82
Infant 17	70.7	39	386	374	2.69	7.49
Infant 18	56	39	418	420	3.67	16.01
Infant 19	57	39	496	509	3.82	20.24
Infant 20	70.2	39	417	385	2.20	4.07
Infant 21	56.5	40	337	343	3.99	31.74
Infant 22	74	39	352	345	1.91	3.20
Infant 23	76.2	39	201	191	1.85	3.25
Infant 24	47.4	40	433	414	3.69	17.85
Infant 25	79.7	40	296	555	1.90	3.12
Infant 26	86.87	39	302	294	1.63	2.77

3.2. Taxonomic Composition of the Bacterial Community

The results indicate that sequences from all samples belonged to four bacterial phyla: Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes. These findings align with previous studies, which identify these phyla as the primary contributors to the establishment of the intestinal microbiota in infants [20]-[22]. This concordance may be due to the focus on infants of a similar age group as those in the referenced studies.

In general, among infants who received complementary foods (BF + FD, FF + FD, BF + FF + FD), Actinobacteria was the most dominant phylum (55%), followed closely by Bacteroidetes (54%). Firmicutes/Bacteroidetes Ratio as an Indicator of Gut Microbiota State. Since Bacteroidetes and Firmicutes are the dominant phyla in the intestine, the ratio between these groups is often considered an informative indicator of the overall state of the intestinal microbiota. Studies have shown that the Firmicutes/Bacteroidetes ratio is lower in the elderly compared to young adults [23]. In this study, the Firmicutes/Bacteroidetes ratio was similarly low, which may be attributed to the early introduction of complementary foods. The intake of these foods produced a gut microbiota profile closer to that of adults, as the addition of complementary foods in infant diets reduced the abundance of

Firmicutes.

Diet and Gut Microbiota Composition

These results indicate that the composition of an individual's gut microbiota is influenced by their diet. Numerous studies have examined the timing of complementary food introduction during the first year of life [24]. Findings from these studies often show a dominance of Firmicutes, followed by Actinobacteria, after food diversification. However, in the present study, diversification resulted in a dominance of Actinobacteria and Bacteroidetes. This difference may be due to the varied ages at which diversification occurred and the specific diets introduced in this study. Additionally, infants in this study received only semiliquid foods, which may have had a milder impact on the gut microbiota compared to more solid or varied diets.

3.3. Actinobacteria Dominance in Different Diets

In the FF + FD diet, Actinobacteria is the dominant phylum at 7%, highlighting its prevalence as diet-dependent. Although these infants began dietary diversification early and were never breastfed, it appears that foods rich in Bifidobacterium are frequently provided to them.

In the BF + FD diets, Actinobacteria dominates at 23%, followed closely by Bacteroidetes (22%) and Firmicutes (21%). The predominance of Actinobacteria suggests that, despite the introduction of complementary foods, the intestinal microbiota of these infants was not profoundly altered. This may be due to the semiliquid nature of these early foods, their irregular introduction, and the continued practice of breastfeeding.

In the BF + FF + FD diets, Bacteroidetes and Firmicutes predominate with 28% each, followed by Actinobacteria at 25%. This balance indicates a notable shift in the gut microbiota composition, likely due to the combined effects of mixed feeding practices and diverse dietary inputs.

3.4. Intestinal Flora Composition by Diet

The four phyla—Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria—are more prevalent in infants on a milk-based diet combined with complementary foods.

A comparative study of infants who began early food introduction (FF + FD, BF + FD, BF + FF + FD) reveals a diverse range of bacterial families. In BF + FD diets, the dominant gut microbiota families are Bifidobacteriaceae (17.38%) and Enterobacteriaceae (9.13%). This dominance is likely due to breastfeeding, as breast milk supplies oligosaccharides to the intestine, promoting the growth of Bifidobacterium species in the ecosystem as long as breastfeeding remains the primary food source. Additionally, breast milk intake is associated with Bifidobacterium presence [25]-[27].

Another possible reason for this composition is that complementary foods had

a limited impact on the gut microbiota in BF + FD infants, likely due to the types of foods introduced and their infrequent administration.

3.5. Microbiota Diversity in Different Diets

In the BF + FD diet, Streptococcaceae represents 7.21%. However, in the feces of infants on this diet, the families Bacteroidaceae, Coriobacteriaceae, Lachnospiraceae, and Lactobacillaceae show higher prevalence than others. The presence of this diverse microbiota suggests a high level of diversity and complexity in infants receiving both breast milk and complementary foods, likely due to the added dietary variety. This complementary feeding appears to reduce the bifidogenic population, and early complementary feeding has been associated with an increased risk of gastrointestinal and respiratory infections [28].

In the FF + FD diet, the proportions of these bacteria decrease, except for Bifidobacteriaceae (23.83%) and Enterobacteriaceae (14.06%), which show an increase. Notably, in the feces of infants on the FF + FD diet, Bacteroidaceae, Coriobacteriaceae, Lachnospiraceae, and Lactobacillaceae are less dominant. These infants, who have not been breastfed, may be consuming formula containing probiotics, explaining the prevalence of the bifidogenic population despite the absence of breast milk.

3.6. Microbiota Composition in BF + FF + FD Diets

In the feces of infants on BF + FF + FD diets, families such as Akkermansiaceae, Atopobiaceae, Bacteroidaceae, Veillonellaceae, Streptococcaceae, Coriobacteriaceae, and Lachnospiraceae are more dominant. This composition reflects the diversity and complexity of the gut microbiota when supplementary foods are introduced, resembling an adult-like microbiota profile.

The abundance of Lachnospiraceae, in particular, is a predictive factor for overweight risk, especially following early dietary diversification between three and four months [29] [30]. Early introduction of complementary foods accelerates the maturation of the gut microbiota in children, aligning it more closely with an adult microbiota profile [31] [32]. However, inadequate microbiota maturation during this period is linked to suboptimal growth and development in early life [33].

Moreover, early food introduction is associated with a higher relative abundance of Akkermansiaceae in infants' stools. While a greater presence of Akkermansiaceae has been linked to improved metabolic health in adults [34] [35], this bacterial family could potentially have adverse effects in infants.

3.7. Microbiota Composition in Various Diets

Furthermore, the families Desulfovibrionaceae and Eggerthellaceae are nearly absent in the feces of children subjected to the different diets (BF + FD, FF + FD, BF + FF + FD). Infants receiving complementary foods exhibit a more complex and diverse intestinal microbiota. Notably, the microbiota of infants on the BF + FF + FD diet is more complex and diverse compared to those on the BF + FD and FF + FD diets.

In each diet group (BF + FD, FF + FD, and BF + FF + FD), 19 bacterial genera were detected, with each representing less than 1% abundance. The genus Escherichia-Shigella is more prevalent in the feces of infants on the FF + FD diet (14.35%) compared to those on the BF + FD (9.37%) and BF + FF + FD diets (9.17%). This indicates a higher presence of pathogenic flora in infants receiving diets supplemented with complementary foods.

The genus Lactobacillus is more abundant in infants on the BF + FD diet (6.63%) than in those on the BF + FF + FD (6.55%) and FF + FD (5.59%) diets. In contrast, the genus Streptococcus is more prevalent in infants on the BF + FF + FD diet (7.68%) compared to BF + FD (7.4%) and FF + FD (6.4%).

Additionally, new bacterial genera, such as Aquamonas, Libanicoccus, Megasphaera, and Eggerthella, were identified, though they are less well-known. The analysis of the intestinal microbiota composition in infants who received different diets revealed a higher abundance of Bifidobacterium in FF + FD infants (24.33%) compared to BF + FD infants (17.84%) and BF + FF + FD infants (14.99%). Moreover, Escherichia-Shigella is significantly more abundant in FF + FD infants (14.35%) than in BF + FD (9.37%) and BF + FF + FD (9.17%) infants. The presence of this pathogenic flora may be linked to the intake of complementary foods and the use of infant formula, particularly in unsanitary conditions (Figures 1-3).

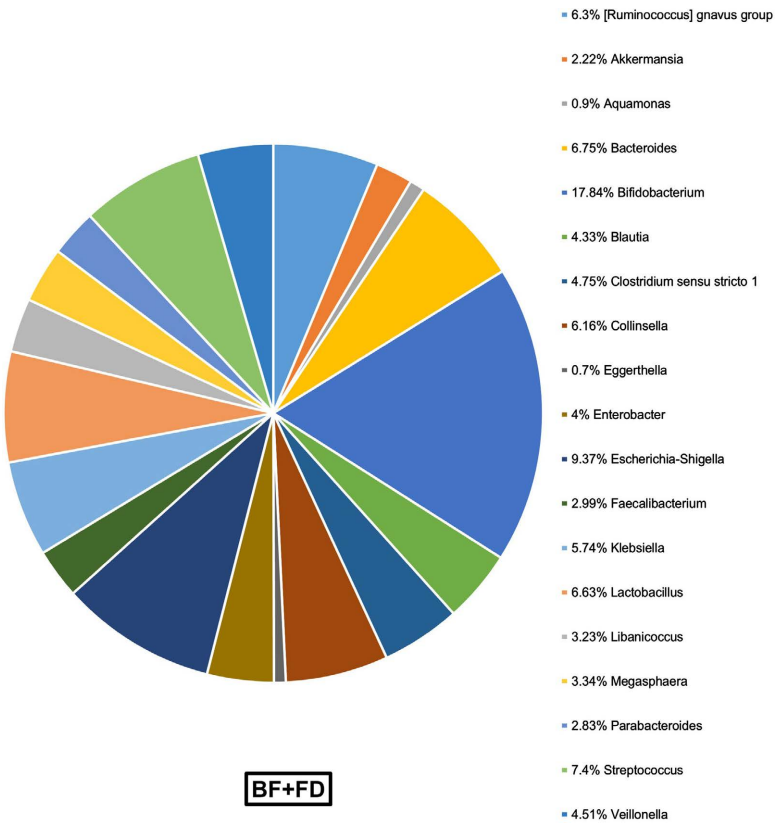

Figure 1. Relative abundance of intestinal flora at the genus level in infants on BF + FD diets.

This figure illustrates the relative abundance of various genera of intestinal flora in infants subjected to and BF + FD diets. The data highlights the differences in microbiota composition between the two dietary groups, showcasing specific genera that are more prevalent in each diet. This comparative analysis provides insights into how dietary choices influence the gut microbiota of infants.

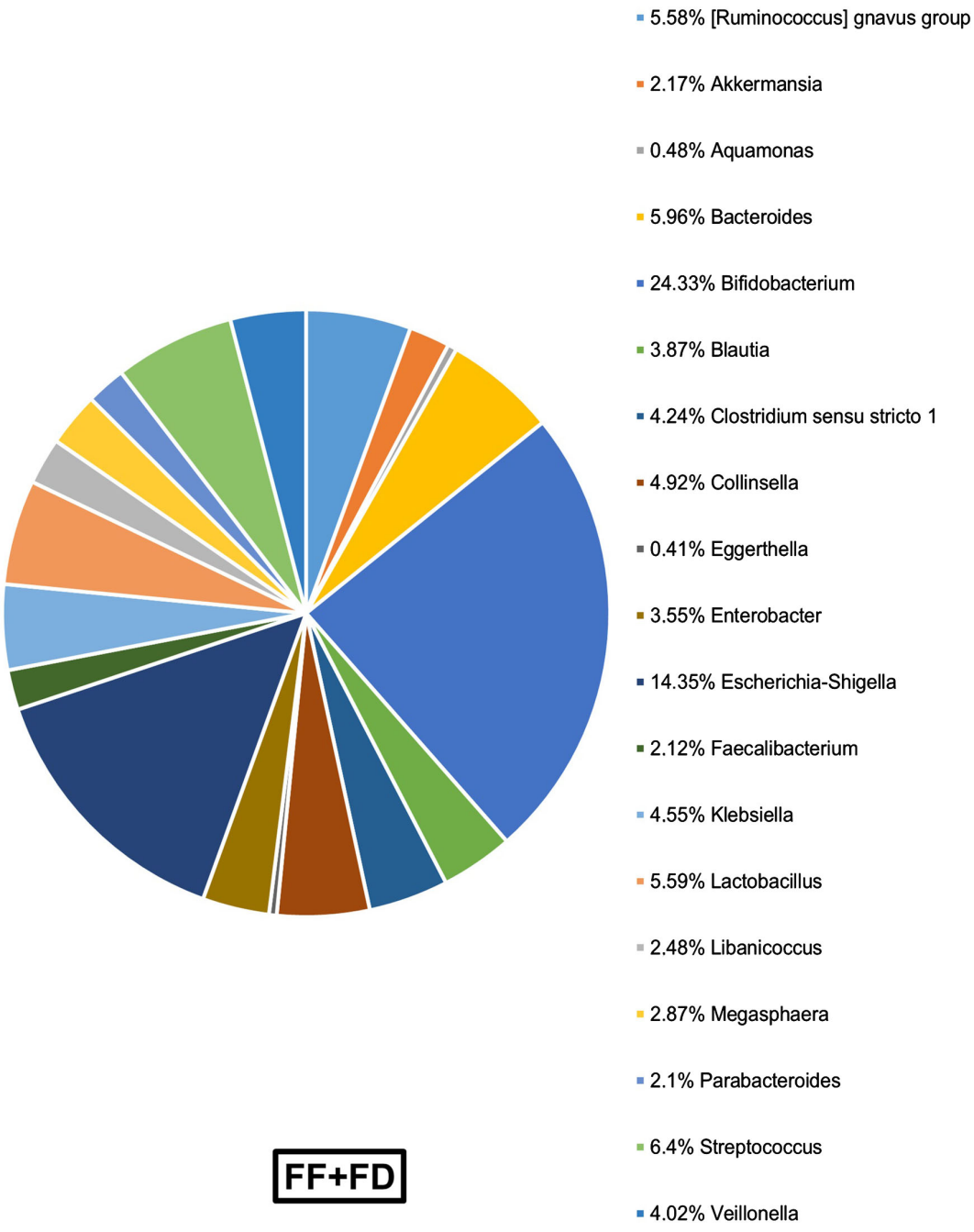

Figure 2. Relative abundance of intestinal flora at the genus level in infants on FF + FD diets.

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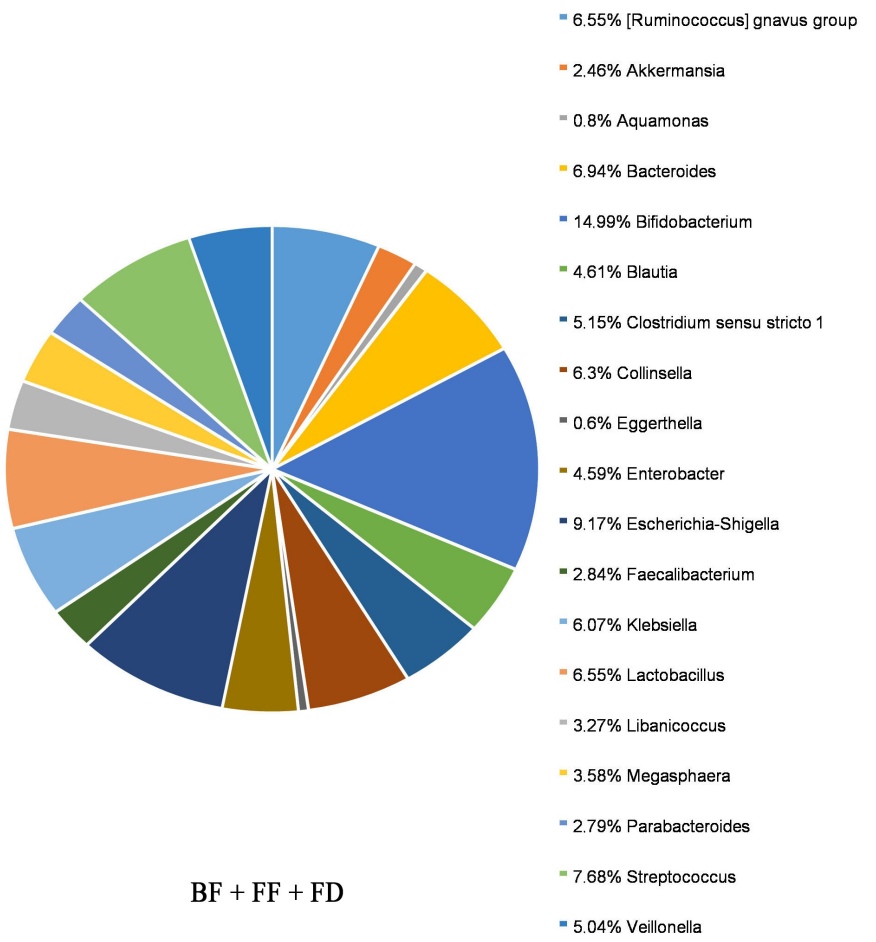


Figure 3. Relative abundance of intestinal flora at the genus level in infants on the BF + FF + FD diet.

This figure presents the relative abundance of various genera of intestinal flora in infants following the BF + FF + FD diet. It illustrates the composition of the gut microbiota within this dietary group, highlighting the specific genera that are predominant. The data provides valuable insights into how the BF + FF + FD diet influences the diversity and richness of the intestinal microbiota in infants.

3.8. Link between the Composition of the Gut Microbiota and the Age of Children

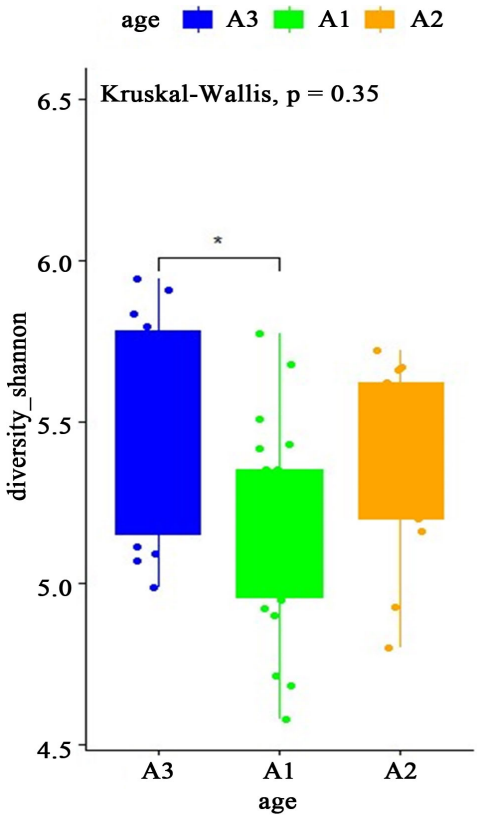
Infants were classified into three age groups: 0 - 119 days, 120 - 179 days, and 180 - 200 days. The analysis of intestinal microbiota composition reveals that **Bifidobacterium** is more dominant in infants aged 120 - 179 days (20.62%) compared to those aged 0 - 119 days (15.7%) and 180 - 200 days (14%). These results suggest that the frequency of breastfeeding may decrease as complementary foods are introduced, leading to a reduction in **Bifidobacterium** abundance.

Enterobacteriaceae levels were highest in infants aged 0 - 119 days, followed by those aged 180 - 200 days, and lowest in the 120 - 179 days group. This variation in abundance is linked to dietary differences among the age groups, aligning with

findings from previous studies indicating higher **Enterobacteriaceae** presence in infants at three months of age 0 - 119 days age group, the intestinal microbiota was characterized by a richness of **Bacteroidaceae** (6.81%), **Bifidobacteriaceae** (15.7%), **Enterobacteriaceae** (11.28%), **Enterococcaceae** (6.09%), **Lactobacillaceae** (6.63%), and **Streptococcaceae** (7.32%). In contrast, less abundant families included **Veillonellaceae** (4.62%), **Ruminococcaceae** (2.67%), **Prevotellaceae** (3.02%), **Desulfovibrionaceae** (0.1%), and **Akkermansiaceae** (2.16%). This microbiota composition indicates that early diversification may influence the gut microbiota of infants in this age range.

Breastfed infants typically exhibit a microbiota rich in **Bifidobacterium** and **Lactobacillus**, with lower levels of ***Escherichia coli*** and ***Clostridium difficile***. However the study found that 60% of infants in the 0 - 119 days age group received complementary foods, suggesting that early food introduction can complicate the expected milk-only microbiota, leading to similarities with an adult-like microbiota.

In the 120 - 179 days age group, the intestinal microbiota showed increased levels of **Bifidobacteriaceae** (20.62%), **Enterobacteriaceae** (8.95%), and **Streptococcaceae** (7.15%), but decreased abundance of **Veillonellaceae** (4.3%), **Enterococcaceae** (5.41%), and **Clostridiaceae** (5.34%). These findings confirm that early introduction of complementary foods is more prevalent among infants aged 0 - 119 days.



Age Groups: A1: 0 - 119 days; **A2**: 120 - 179 days; **A3**: 180 - 200 days; *Indicates significantly different (*p < 0.05).

Figure 4. Indexes of infant age diversity.

The **Lactobacillaceae** family was predominant in infants aged 180 - 200 days (6.69%) compared to those aged 0 - 119 days (6.63%) and 120 - 179 days (5.6%). The presence of **Lactobacillus** in this age group suggests an association with formula feeding. The higher prevalence of **Lactobacillus** could also indicate increased consumption of probiotic-rich foods or a return to exclusive breastfeeding practices by mothers. Overall, the gut microbiota of infants receiving early complementary foods tends to resemble that of adults, characterized by a dominance of **Bacteroidetes**, **Firmicutes** (including **Enterococcus** and **Streptococcus**), **Actinobacteria** (primarily **Bifidobacterium**), and **Proteobacteria**. This microbiota exhibits considerable interpersonal and intrapersonal variability. The relationship between microbiota composition and age is complex, with studies showing an increase in facultative anaerobes such as Enterococcus and Streptococcus with aging. Conversely, some studies report a decline with age, while others show no significant reduction until the elderly population. A significant difference in gut microbiota composition was observed across age groups (Wallis test, $p < 0.05$).

Figure 4 visually represents the diversity indexes of the gut microbiota across different infant age groups, highlighting significant variations in microbial composition based on age.

3.9. Long-Term Impacts of Early Microbiota on Immune Development

The early period is crucial for establishing a diverse and balanced gut microbiota, which plays a central role in the maturation of the immune system. Early alterations in microbiota composition (e.g., due to inadequate nutrition or excessive antibiotic use) can increase the risk of chronic inflammatory diseases, such as asthma, allergies, and autoimmune disorders. **Metabolism and Obesity:** An imbalanced gut microbiota (dysbiosis) in young children has been linked to an increased risk of obesity and metabolic syndrome in adulthood. Specific bacteria influence nutrient absorption and fat storage. **Mental Health:** The gut-brain axis indicates that the gut microbiota influences neurological and behavioral development. Early disruptions could be associated with neurodevelopmental disorders such as autism or mood disorders. **Predisposition to Chronic Diseases:** Altered gut microbiota can affect susceptibility to cardiovascular diseases, type 2 diabetes, and even certain cancers.

This study could not only contribute to a better understanding of the impacts of early dietary diversification on the gut microbiota but also inform concrete interventions to improve public health.

4. Conclusions

The present study demonstrated significant differences in the composition of the intestinal microbiota based on the various diets provided to infants ($p = 0.05$). Additionally, there was a notable difference in the gut microbiota composition

across different age groups ($p < 0.05$). The analysis revealed that Actinobacteria and Bacteroidetes were the most dominant phyla in all three dietary groups: BF + FD, FF + FD, and BF + FF + FD. Conversely, Firmicutes and Bacteroidetes were less represented in infants on FF + FD diets. There is variability in the composition of the intestinal flora among children who consumed foods other than milk. In terms of age-related findings, Bifidobacterium and Enterobacteriaceae were the predominant bacteria in infants aged 0 - 119 days. This dominance continued in the 120 - 179 days group, albeit with reduced proportions. By the age of 180 - 200 days, both Bifidobacterium and Enterobacteriaceae were present in even lower amounts.

Overall, the composition of the microbial community in the feces of each infant reflected slight variations according to diet, indicating that the provided foods had some impact on the intestinal microbiota, although this impact was less profound due to the semi-liquid nature of the foods. In contrast, the gut microbiota composition was significantly influenced by the age of the infants, highlighting its age-dependent nature.

This study contributes valuable insights into the composition of the intestinal microbiota in infants who received early complementary foods within the Ivorian context. Future research should further explore the impact of solid foods on gut microbiota composition and address issues related to early dietary diversification and child malnutrition in Côte d'Ivoire.

5. Limits of the Study

Below are potential limitations relevant to studies investigating the impacts of early dietary diversification on the gut microbiota:

1) Sample Size and Diversity

- **Limited sample size:** A small number of participants might reduce the statistical power and generalizability of the findings.
- **Population homogeneity:** If the study participants come from similar geographic, socioeconomic, or cultural backgrounds, the findings may not be representative of broader populations.

2) Methodological Constraints

- **Cross-sectional design:** Studies capturing data at a single point in time may not establish causal relationships. A longitudinal approach would provide better insights into changes over time.
- **Self-reported dietary data:** Reliance on caregivers' recall or reporting for dietary habits might introduce reporting bias or inaccuracies.

3) Microbiota Analysis Techniques

- **Resolution of sequencing methods:** Some microbiota analysis methods (e.g., 16S rRNA sequencing) may lack the resolution to differentiate closely related bacterial species or to identify functional attributes.
- **Storage and handling of samples:** Variability in sample collection, storage, or processing might affect microbiota composition and introduce biases.

4) Environmental and Confounding Factors

- **Antibiotic use:** Variations in antibiotic exposure among participants could influence microbiota composition independently of dietary factors.
- **Breastfeeding practices:** Differences in breastfeeding duration or exclusivity may confound the relationship between dietary diversification and gut microbiota changes.

5) Lack of Long-Term Follow-Up

- Without follow-up into later childhood or adulthood, the study may not capture the long-term implications of early dietary impacts on health outcomes.

6) Geographical and Cultural Specificity

- The study's findings may reflect unique dietary or environmental conditions of the population studied, limiting their applicability to other settings with different food practices or healthcare access.

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Conflicts of Interest

There is no financial conflict of interest related to the work described in this study.

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