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REVIEW ARTICLE

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The effect of complementary foods on the colonic microbiota of weaning infants: a systematic review

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ABSTRACT

The transition from breastmilk to solid foods (weaning) is a decisive stage for the development of the colonic microbiota. However, little is known about how complementary foods influence the composition and function of the colonic microbiota in infants. This systematic review collected evidence of the effect of individual foods on the fecal microbiota of weaning infants (4–12 months old) using five databases: PubMed, CENTRAL, Scopus, Web of Science, and ScienceDirect. A total of 3625 records were examined, and seven randomized clinical trials met the review's eligibility criteria. Altogether, 983 participants were enrolled, and plant-based foods, meats, and dairy products were used as interventions. Wholegrain cereal increased the fecal abundance of the order Bacteroidales in the two included studies. Pureed beef increased the fecal abundances of the genus *Bacteroides* and the *Clostridium* XIVa group, as well as microbial richness in two of the three included studies. However, the conclusions of this review are limited by the small number of studies included. No conclusions could be drawn about the impact of complementary foods on fecal metabolites. Further clinical trials assessing the effect of dietary interventions on both fecal microbial composition and function are needed to fill this knowledge gap in infant nutrition.

KEYWORDS
Infant; colonic microbiota; complementary foods; weaning

1. Introduction

The human colon is home to a diverse microbial community called the colonic microbiota. Microbes from the mother's vaginal canal and feces are the first to populate the newborn's colon, followed by microbes found in the environment (Ferretti et al. 2018). In human studies, the colonic microbiota is mainly characterized using fecal samples (Hsieh et al. 2016). Multiple factors belonging to the host and the environment continuously shape the fecal microbiota during infancy (Stewart et al. 2018; Roswall et al. 2021). According to this complex and not yet fully understood interplay of factors, colonic commensals influence the host's physiology. A balanced fecal microbiota is linked with disease prevention and overall longevity (Singh et al. 2019; Manor et al. 2020). Conversely, imbalances in the microbiota or dysbiosis are associated with gastrointestinal and metabolic diseases, among other pathologies (Frank et al. 2007; Zhao et al. 2020).

In the early stages of life, breastmilk promotes the growth of microbes capable of metabolizing human milk oligosaccharides, notably bifidobacteria (Marcobal et al. 2010). At around 6 months of age, the introduction of complementary foods and the simultaneous reduction of breastmilk intake (weaning) create conditions for other microbes to thrive (Laursen et al. 2016; Homann et al. 2021). At this stage, the infant's gastrointestinal system is not yet fully developed (Fournier et al. 2022). Macronutrients from complementary foods, especially complex carbohydrates and proteins, can reach the colon undigested. This promotes the growth of a myriad of new colonic commensals specialized in degrading these nutrients to produce, among other metabolites, short-chain fatty acids (SCFAs), like the genera *Bacteroides*, *Clostridium*, *Prevotella*, and *Ruminococcus* (Bäckhed et al. 2015; Stewart et al. 2018; Gómez-Martín et al. 2022).

Dietary changes during the weaning period gradually increase fecal microbial richness and diversity, supporting the complete maturation of the fecal microbiota, which typically stabilizes by 2 to 3 years of age (Yatsunenko et al. 2012; Wernroth et al. 2022). At this stage, the infant microbiota is more stable, resembling the adult microbiota in composition and function (Yatsunenko et al. 2012). Acetate, propionate, and butyrate become the major SCFAs produced by colonic microbes, primarily contributing to gut health while also providing other benefits to the host (Liu et al. 2021). Changes

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in the fecal microbiota that occur during weaning may persist for the long term, ultimately influencing host health later in life (Arrieta et al. 2015; Beller et al. 2021). Therefore, weaning represents a unique opportunity to establish beneficial interactions between the host and its colonic microbes.

Nevertheless, microbiota investigations traditionally focus on early infancy or adulthood, neglecting the weaning period. Limited knowledge exists about the effects of individual complementary foods on the fecal microbiota of weaning infants, and the question of the best complementary feeding practices for nourishing colonic commensals in this crucial stage of life remains unanswered.

This systematic review investigated how complementary foods affect the composition and function of the fecal microbiota of infants (4–12 months old) to shed light on this knowledge gap in infant nutrition. Changes in fecal microbial diversity, relative abundance of bacterial taxa, and short-chain fatty acids (SFCAs) and branched-chain fatty acids (BCFAs) concentrations were identified in response to clinical interventions with complementary foods.

2. Materials and methods

2.1. Search strategy

This systematic review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher et al. 2009) and was registered on PROSPERO (CRD42023438679). The eligibility of studies was assessed using the PICOS (Population, Intervention, Comparator, Outcome, and Study Design) criteria (Table 1).

Studies were identified using five databases: PubMed, CENTRAL, Scopus, Web of Science, and ScienceDirect. Search terms were related to food, infant, weaning, and microbiota, as follows: (food OR diet OR feeding OR nutrition) AND (bowel OR gut OR colon* OR feces OR faeces OR fecal OR faecal OR gastrointestinal OR intestinal OR stool) AND (microb* OR bacteria OR microorganism* OR micro-organism*) AND (infancy OR "early life" OR "early-life" OR infant* OR baby OR babies OR toddler* OR child*) AND (weaning OR complementary OR complimentary OR supplementary).

2.2. Eligibility criteria

The search strategy was tailored to each database to identify articles in English published up to October 2024 (Supplementary Table 1). The inclusion criteria were randomized controlled trials and cross-over studies that reported the effect of complementary foods on the colonic microbiota of healthy infants at weaning age (4–12 months old) as primary or secondary outcomes. The main outcomes were changes in the fecal microbial composition and/or production of microbial organic acids, measured by comparing the intervention group with the control group at the end of the intervention.

Table 1. PICOS criteria for inclusion of studies.

Parameter	Criteria
Population	Healthy infants at weaning age (4 to 12 months old)
Intervention	Use of any complementary food
Comparator	Placebo control or any other complementary food used as control
	Participants not exposed to the intervention or consuming their habitual diets
Outcome	Changes in colonic microbiota composition (alpha and beta diversity scores, relative abundance of bacterial taxa) and/or function (production of short-chain or branched-chain fatty acids)
Study design	Randomized controlled trials

No restrictions were placed on the infant's mode of delivery (vaginal or cesarean delivery), feeding strategy before the introduction of solids (breastmilk or infant formulas), geographical location, and ethnicity. Studies were excluded if the infants were unhealthy(diagnosed with chronic diseases, including gastrointestinal, metabolic, or neurological conditions) or not at the defined age. Interventions with supplements containing prebiotics or probiotics, isolated food compounds, and food additives were excluded. Alternatively, trials evaluating foods that naturally contain prebiotics or probiotics (for instance fermented foods) were included. Studies evaluating the impact of infant formulas on the colonic microbiota of infants were excluded. Systematic and narrative reviews were also excluded.

2.3. Data extraction

Records were identified using the described search strategy. Titles and abstracts of studies were independently screened by two review authors (VG, JT) using the web application PICO Portal (PICO Portal, USA). Studies that potentially meet the inclusion criteria were selected for full-text eligibility assessment, which was independently performed by two reviewers (VG, JT). Disagreements in judgment were resolved by discussion with a third reviewer (WM). A standardized form was used to extract data from the included studies by two independent reviewers (VG, JT). Extracted information included study design (length, sample size, sample collection points, methods), study population (age, nationality, gender, inclusion criteria), details of the intervention (food intervention, quantity, control), and outcomes (changes in alpha and beta diversity scores, changes in the relative abundance of microbial taxa, production of SCFAs and BCFAs.

2.4. Risk of bias assessment

The risk of bias in selected studies was independently assessed by two reviewers (VG, JT) using the Cochrane risk-of-bias tool for randomized trials (RoB2) (Sterne et al. 2019). The following elements of the studies were assessed: the randomization process, deviations from the intended interventions, missing outcome data, a measure of the outcome, and selective reporting of results. Scores for each element were generated by the Rob2 algorithm, resulting in "low risk of bias," "some concerns," or "high risk of bias." Conflicting scores between reviewers were resolved by discussion to reach a consensus.

3. Results

3.1. Characteristics of identified studies

A total of 3625 unique studies were identified after removing duplicates, of which 3618 were excluded for various reasons (Figure 1), such as not meeting age and health criteria, use of probiotics and/or prebiotics, inappropriate study design, or lack of a discernible independent effect of complementary foods on the infant fecal microbiota. Of these, seven food intervention trials met the eligibility criteria and were included in this systematic review: six randomized controlled trials (Krebs et al. 2013; Qasem et al. 2017; Zambrana et al. 2019; Ordiz et al. 2020; Bierut et al. 2021; Tang et al. 2023;) and one randomized cross-over trial (Plaza-Diaz et al. 2021).

Study characteristics are summarized in Table 2. A total of 983 infants were enrolled in the identified studies (enrollment ranged from 45 to 355 participants) and their mean age at enrollment was approximately 6 months, ranging from 4 to 9 months old. Trial intervention times ranged from 2 weeks to 7 months, 3 months being the most common trial length (3 out of 7 trials). Studies were conducted in Africa (Mali and Malawi), North America (USA and Canada), Europe (Spain) and Central America (Nicaragua), with one study conducted simultaneously in two different countries: Nicaragua and Mali (Zambrana et al. 2019).

The majority of the identified trials (5 out of 7) used plant-based foods as an intervention (Krebs et al. 2013; Qasem et al. 2017; Zambrana et al. 2019; Ordiz et al. 2020; Plaza-Diaz et al. 2021), including legumes (beans and cowpea, which is also known as black-eyed peas), rice bran, and commercial cereal products. Two studies used whole-grain cereals as an intervention (Krebs et al. 2013; Plaza-Diaz et al. 2021). The effect of pureed meats (beef, pork, and poultry) on the fecal microbiota of infants was assessed by three studies (Krebs et al. 2013; Qasem et al. 2017; Tang et al. 2023) while dairy products (yoghurt, cheese, whey protein, and bovine colostrum) were used in two trials (Bierut et al. 2021; Tang et al. 2023). One study assessed the combination of bovine colostrum with egg powder (Bierut et al. 2021). In most studies (4 out of 7), other intervention products were used as control (Krebs et al. 2013; Qasem et al. 2017; Plaza-Diaz et al. 2021; Tang et al. 2023), while two trials used a mixture of corn-soy flour (Ordiz et al. 2020; Bierut et al. 2021) and one study used the absence of an intervention (Zambrana et al. 2019).

All the identified studies used feces to represent the colonic microbiota of infants. Fecal samples were collected at least three times in most of the trials (4 out of 7) (Krebs et al. 2013; Zambrana et al. 2019; Ordiz et al. 2020; Tang et al. 2023;), while three studies collected the samples only at baseline and at the end of the intervention (Qasem et al. 2017; Bierut et al. 2021; Plaza-Diaz et al. 2021). The fecal microbiota composition was determined by 16S ribosomal RNA amplicon sequencing in all studies, mainly through amplifying the V3–V4 regions, while one study amplified all nine variable regions (Bierut et al. 2021). Bioinformatic tools varied between studies; none used the same pipeline and reference database. QIIME (Kuczynski et al. 2011) and its updated version, QIIME2(Bolyen et al. 2019), were the most commonly used pipeline (3 out of 7 trials) (Qasem et al. 2017; Ordiz et al. 2020; Plaza-Diaz et al. 2021), while SILVA (Quast et al. 2013) was the most employed reference database (4 out of 7 trials) (Zambrana et al. 2019; Bierut et al. 2021; Plaza-Diaz et al. 2021; Tang et al. 2023;).

Among the seven trials, only two assessed the metabolite production of the fecal microbiota (Zambrana et al. 2019; Tang et al. 2023;). Zambrana and coauthors (2019) characterized the non-targeted metabolite profile of fecal samples at 2 months of intervention by ultrahigh-performance liquid chromatography-tandem mass spectrometry. Tang et al. (2023) measured fecal SCFAs and BCFAs at three different time points using gas chromatography-mass spectrometry. Out of the seven identified studies, three were found to have some concerns of bias (Krebs et al. 2013; Qasem et al. 2017; Zambrana et al. 2019), three had a low risk of bias (Bierut et al. 2021; Plaza-Diaz et al. 2021; Tang et al. 2023), and one had a high risk of bias (Ordiz et al. 2020) (Table 2). The main source of bias in most studies was potential selection in reporting results, which corresponds to domain five of the RoB2 tool (Supplementary Figure 1).

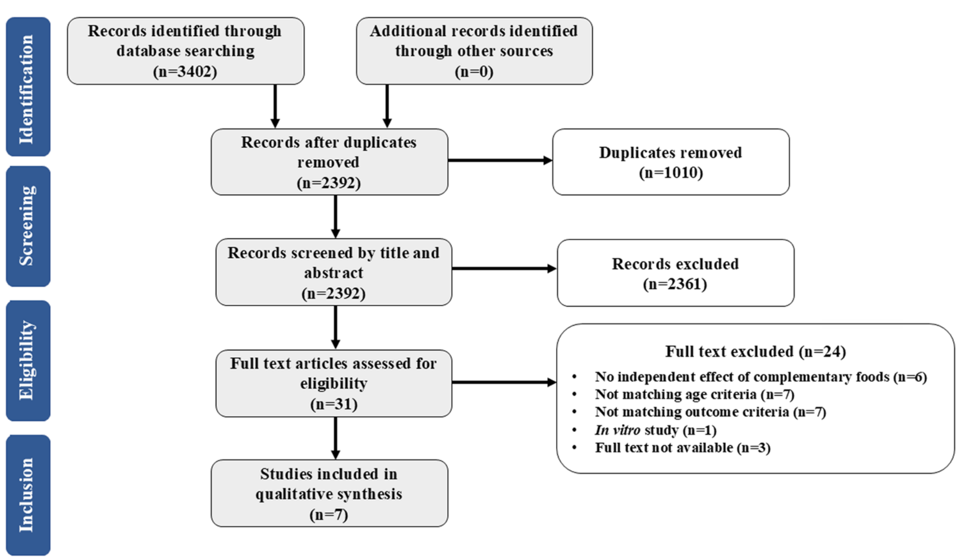

Figure 1. PRISMA flow diagram of literature search.

Table 2. Characteristics of identified studies.

References	Study design	Intervention	Population	Inclusion criteria	Antibiotic usage during the trial	Infant diet during the trial	Dietary intake assessment	Microbiota analyses	Risk of bias
Bierut et al. (2021)	Randomized, blinded, placebo-controlled trial (3-month intervention time). Collection of fecal samples at baseline and at the end of the intervention (9 and 12 months of age)	Bovine colostrum (5.7 g) with egg powder (4.3 g) twice daily. Corn/soy flour (15 g) was used as a control	Malawian healthy breastfed infants (9–12 months old, 41% female) ($n=277$ enrolled)	Infants aged 9 months old living in the Limera and Masenjere village clusters without acute malnutrition or other medical conditions affecting normal growth	Not reported	It is unknown whether infants consumed other foods, breastmilk, or infant formula during the trial	Survey of animal source food consumption and use of food supplements for malnutrition. Assessment at the entry of the study, during the intervention (weeks 2, 4, 8, and 12), and after the intervention (weeks 20 and 32)	DNA sequencing by 16S ribosomal RNA (all 9 variable regions) using Illumina MiSeq platform. Bioinformatics using MVRSION pipeline and SILVA 132 database ($n=263$ analyzed at the end of the intervention)	Low risk
Krebs et al. (2013)	Randomized controlled trial (3-month intervention time). Collection of fecal samples at 5, 6, 7, 8, and 9 months of age	Wholegrain iron-fortified cereal, iron and zin-fortified cereal, or pureed beef (1–2 servings/day)	American healthy breastfed infants (5–9 months old, 60% female) ($n=45$ enrolled)	Infants from the Denver metropolitan region, with a gestational age of 37–42 wks, vaginally delivered, exclusively breastfed, without chronic conditions, and aged between 5–6 months old	Not reported	In addition to the intervention, fruits, vegetables, teething biscuits, and unfortified cereals were allowed ad libitum. There was no consumption of infant formula	Use of a 3-day food record questionnaire. Monthly assessment	DNA sequencing by 16S ribosomal RNA (V1-V3 regions) pyrosequencing using a genome sequencer FLX System. Bioinformatics using the Infernal RNA alignment tool and the RDP classifier database ($n=14$ analyzed at 5 to 9 months of age)	Some concerns
Ordiz et al. (2020)	Randomized, double-blind, controlled trial (6-month intervention time). Collection of fecal samples at the baseline, 6.5, 7.5, 9, 10.5, and 12 months of age	Common bean or cowpea (80–120 kcal/d). Corn-soy blend flour (80–120 kcal/d) was used as a control	Malawian healthy breastfed infants (6–12 months old, 48% female) ($n=355$ enrolled)	Healthy infants aged between 5.5 and 6.5 months old living in the Limera and Masenjere village clusters without acute malnutrition or chronic noninfectious diseases	103 courses of antibiotics were recorded (22% in infants from the Limela and 81% in infants from Masenjere villages). It was not reported if there was different antibiotic usage between interventions and controls	In addition to the intervention, infants were breastfed ad libitum and consumed corn porridge as the primary complementary food. It is unknown if they consumed infant formula	Two 24h dietary recalls were conducted in a random subset of 50 participants	DNA sequencing by 16S ribosomal RNA (V4 region) using Illumina MiSeq platform. Bioinformatics using QIIME pipeline and Greengenes 13.8 database ($n=236$ analyzed at ≤ 6-time points)	High risk
Plaza-Diaz et al. (2021)	Randomized, triple-blind, cross-over trial (14-week intervention time). Collection of fecal samples at the baseline and the end of the intervention (7 wks)	50% wholegrain low sugar (12 g/100g) cereal or 0% wholegrain high sugar (24 g/100g) cereal. Parents decided how much cereal to consume	Spanish healthy infants (4–7 months old, 48% female) ($n=48$ enrolled)	Infants with a gestational age of 37–42 wks, weight at birth >2500 g, aged between 4–5 months, not breastfed since at least 4 months old, not yet introduced to complementary foods, and without antibiotic usage in the last 15 days	No use of antibiotics during the intervention period	In addition to the intervention, infants consumed infant formula and other foods. There was no consumption of human milk	Two-non-consecutive-day weighed dietary record. Assessment at baseline and after 1, 4, and 7 wks of intervention	DNA sequencing by 16S ribosomal RNA (V3–V4 regions) using Illumina MiSeq platform. Bioinformatics using QIIME2 and DADA2 pipelines and SILVA 132 database ($n=43$ analyzed at the baseline and 7 wks of intervention)	Low risk

(*Continued*)

Table 2. Continued.

References	Study design	Intervention	Population	Inclusion criteria	Antibiotic usage during the trial	Infant diet during the trial	Dietary intake assessment	Microbiota analyses	Risk of bias
Qasem et al. (2017)	Randomized controlled trial (2–4 weeks intervention time according to parent's discretion). Collection of fecal samples at the baseline and the end of the intervention	Iron-fortified cereal, iron-fortified cereal with raspberry, or pureed beef. The consumed amount of intervention was not reported.	Canadian healthy breastfed infants (4–6 months old, 50% female) ($n = 87$ enrolled)	Infants aged between 4–6 months old with birth weight > 2500 g and more than 37 gestational weeks, exclusively breastfed, not yet introduced to solid foods, and without medical conditions	Not reported	In addition to the intervention, infants consumed breastmilk, but it is unknown whether they consumed infant formula or other foods	Use of a 3-day food record questionnaire	DNA sequencing by 16S ribosomal RNA (V3 to V4 regions) using Illumina MiSeq platform. Bioinformatics using QIIME pipeline and Greengenes 13.5 database ($n = 56$ analyzed at the baseline and the end of intervention)	Some concerns
Tang et al. (2023)	Randomized controlled trial (7-month intervention time). Collection of fecal samples at 5, 10, and 12 months of age	Meat (pureed beef, pork, and poultry) or dairy-based (yoghurt, cheese, and whey protein powder) foods (3 g/kg/day)	American healthy formula-fed infants (5–12 months old, 50% female) ($n = 71$ enrolled)	Infants from Denver (Colorado, USA), full-term, exclusively formula-fed, aged between 3–5 months old, and without chronic diseases	Two infants had antibiotics during the intervention (one from each group)	In addition to the intervention, infants consumed infant formula, cereal, fruit, and vegetables ad libitum. There was no consumption of breastmilk	Use of a 3-day food record questionnaire. Assessments at 5, 10, and 12 months of age	DNA sequencing by 16S ribosomal RNA (V3 to V4 regions) using Illumina MiSeq platform. Bioinformatics using SINA pipeline and SILVA 115 database ($n = 59$ analyzed at 5 months and $n = 57$ at 12 months of age). SCFAs analysis by GC-MS	Low risk
Zambrana et al. (2019)	Randomized controlled trial (6-month intervention time). Collection of fecal samples at 6, 8, and 12 months of age	Rice bran (1–5 g/day). The absence of the intervention was used as a control	Nicaraguan and Malian healthy infants (6–12 months old, 45% and 50% female, respectively) ($n = 50$ and 50 enrolled, respectively). Malian infants were breastfed. 96% of Nicaraguan infants in the control group and 83% in the rice bran group were breastfed	Infants aged between 4 and 5 months old, without diarrhea episodes and antibiotics used in the last month, without prior hospitalizations, allergies, or immune-compromising conditions	Of the 47 Nicaraguan infants, 25 used antibiotics (14 in the control and 11 in the intervention group). Of the 48 Malian infants, 27 used antibiotics (14 in the control and 13 in the intervention group)	In addition to the intervention, infants consumed other foods like staple grain porridges, soups, milk, fruits, juices, eggs, and fish. Nicaraguan infants also consumed breastmilk and infant formula, while Malian infants only breastmilk during the study	Daily dietary records (measuring the consumption of the intervention as none, half, or all)	DNA sequencing by 16S ribosomal RNA (V4 region) using Illumina MiSeq sequencer. Bioinformatics using mothurpipeline and SILVA 128 database ($n = 48$ Malian and 47 Nicaraguan analyzed at 8 and 12 months of age). Metabolome analysis by UPLC-MS (at 8 months of age)	Some concerns

DADA: Divisive Amplicon Denoising Algorithm; GC: gas chromatography; MS: mass spectrometry; MVRSION: Multiple 16S Variable Region Species-Level IdentificatiON; QIIME: Quantitative Insights Into Microbial Ecology; SCFAs: short-chain fatty acids; SINA: SILVA Incremental Aligner; UPLC: ultrahigh-performance liquid chromatography; RDP: Ribosomal Database Project.

3.2. Influence of plant-based foods on the fecal microbiota of weaning infants

Commercial cereals were the most used plant-based foods, being used as intervention products in three of the identified trials (Krebs et al. 2013; Qasem et al. 2017; Plaza-Diaz et al. 2021). Wholegrain cereals were used in two studies (Krebs et al. 2013; Plaza-Diaz et al. 2021). Plaza-Diaz et al. (2021) evaluated the influence of cereals made with wheat, corn, rice, oats, barley, rye, sorghum, and millet on the fecal microbiota of Spanish infants not breastfed since at least 4 months of age. Two formulations were used during seven weeks of intervention, one rich in wholegrains (50%) and the other rich in sugar (0% wholegrain and 24 g of sugar/100g). In addition to the intervention, infants consumed infant formula and other foods. There was no consumption of human milk. Of the 48 enrolled participants, 43 had their fecal microbiota analyzed, 18 in the sugar-rich cereal group and 25 in the whole-grain cereal group. Infants did not use antibiotics during the intervention period. A permutational multivariate analysis of variance demonstrated that the cereal type had a small but significant effect on the fecal microbial composition of samples (p-value = 0.029), explaining 2% of the variance between samples. After the intervention, infants who consumed whole-grain cereal had a lower relative abundance of the *Escherichia-Shigella* genus compared to those who consumed sugar-rich cereal. Longitudinal changes in the relative abundance of microbial taxa were also observed. The whole-grain cereal group showed an increase in the relative abundances of *Bacteroides* and *Lachnoclostridium* genera compared to the baseline. Both intervention groups had a decrease in the abundance of the *Enterococcus* genus and an increase in the abundance of the *Veillonella* genus by the end of the intervention. However, there were no differences in fecal microbiota alpha diversity scores when comparing the end of the intervention values to the baseline values for both cereal groups (Table 3).

In a 3-month trial, Krebs and colleagues (2013) allocated exclusively breastfed American infants(5 months old) to either wholegrain iron-fortified cereal, iron- and zinc-fortified cereal, or pureed beef. In addition to the intervention, fruits, vegetables, teething biscuits, and unfortified cereals were allowed ad libitum. There was no consumption of infant formula. The study monitored the use of antibiotics during the trial but did not report it. Fecal samples were collected and analyzed monthly for a small subset of 14 infants (out of 45 enrolled), with four in the wholegrain cereal, six in the zinc-fortified cereal, and four in the pureed beef group. Despite the small sample size, significant changes (p-value < 0.05) in microbial composition were observed between the intervention groups at the end of the study. Infants consuming wholegrain iron-fortified cereal had reduced abundances of the *Bifidobacterium* genus and the Lactobacillales order and an increased abundance of the Bacteroidales order compared to the other infants (Table 3). No longitudinal changes in the Chao1 species richness estimator were observed for any of the three intervention groups.

In a similar study, Qasem and coauthors (2017) evaluated the influence of iron-fortified rice cereal, iron-fortified rice cereal with raspberry, or pureed beef on the fecal microbiota of exclusively breastfed Canadian infants(4 to 6 months old). In addition to the intervention, infants consumed breastmilk, but it is unknown whether they consumed infant formula or other foods. The trial lasted from 2 to 4 weeks according to the parents' discretion. Of the 87 enrolled infants, 82 completed the study, and 56 had their fecal microbiota analyzed (18 in the iron-fortified cereal, 19 in the iron-fortified cereal with raspberry, and 19 in the pureed beef group). The use of antibiotics during the intervention was not reported. In contrast to the findings of Krebs et al. (2013), there were no differences in the microbial composition between intervention groups (permutational analysis of variance, p-value = 0.22) nor did it find longitudinal changes in the relative abundance of microbial taxa after correction for multiple comparisons. On the other hand, the Chao1 richness index increased for the iron-fortified rice cereal with raspberry and pureed beef groups at the end of the intervention (Table 3).

Ordiz and colleagues (2020) evaluated the effect of a 6-month legume supplementation on the fecal microbiota of breastfed Malawian infants. In addition to the intervention, infants were breastfed ad libitum and consumed corn porridge as the primary complementary food. It is unknown if they consumed infant formula. The trial recruited 355 participants and divided them into three intervention groups: common bean, cowpea, or a mixture of corn and soy flour (control). The use of antibiotics was recorded (103 times), but it was not reported if there was different antibiotic usage between interventions and controls. Of the 291 infants who completed the trial, 236 had their fecal microbiota analyzed (82 in the cowpea group, 78 in the common bean group, and 76 in the control group). No changes in Faith's Phylogenetic Diversity or weighted UniFrac distances were observed between groups at the end of the study, nor were longitudinal changes in diversity scores for each intervention. However, infants who consumed cowpea had increased relative abundances of the genus *Bifidobacterium* and decreased abundances of the genus *Prevotella* compared to those who consumed common bean or corn-soy flour.

Zambrana et al. (2019) studied the impact of rice bran on the fecal microbiota of predominantly breastfed 6-month-old infants from Nicaragua and Mali. In addition to the intervention, infants consumed other foods like staple grain porridges, soups, milk, fruits, juices, eggs, and fish. Nicaraguan infants also consumed breastmilk and infant formula, while Malian infants only breastmilk during the study. Of the 100 enrolled participants (50 in each country), 47 Nicaraguan and 48 Malian infants completed the 6-month intervention. Notably, 52 infants received antibiotics during the trial: 25 Nicaraguan infants (14 in the control and 11 in the intervention group) and 27 Malian infants (14 in the control and 13 in the intervention group). No changes in alpha diversity indices (Observed, Shannon, Inverse Simpson, and Richness) between intervention and control groups were observed for either cohort. However, infants from different locations had distinct microbiota compositions at 8 and 12 months of age (the fecal microbiota was not measured at the study's baseline). At the end of the intervention(12 months old), Nicaraguan infants that consumed rice bran had increased

Table 3. Changes in the fecal microbiota composition and function according to complementary food intervention.

		Microbiota composition				Microbiota function
		Changes between baseline and at the end of the intervention		Changes between groups at the end of the intervention		
Reference	Complementary food	Diversity indexes	Relative abundance (order, family, or genus)	Diversity indexes	Relative abundance (order, family, or genus)	Short-chain and branched-chain fatty acids
Plaza-Diaz et al. (2021)	0% wholegrain high-sugar cereal	No changes in Shannon, Pielou's evenness, Simpson's, and inverse Simpson's indices	↑*Veillonella* ↓*Bifidobacterium, Enterococcus*	No changes in Shannon, Pielou's evenness, Simpson's, and inverse Simpson's indices between groups	↓*Escherichia-Shigella* (wholegrain cereal compared to high-sugar cereal)	Not evaluated
	50% wholegrain, low-sugar cereal	No changes in Shannon, Pielou's evenness, Simpson's, and inverse Simpson's indices	↑*Veillonella, Bacteroides, Lachnoclostridium* ↓*Escherichia-Shigella, Enterococcus*			Not evaluated
Krebs et al. (2013)	Wholegrain iron-fortified cereal	No changes in the Schao1 diversity index	↑Bacteroidales, *Clostridium* group XIVa ↓*Bifidobacterium,* Lactobacillales, *Enterobacteriaceae*	No changes in the Schao1 diversity index between groups	↑Bacteroidales ↓*Bifidobacterium, Rothia,* Lactobacillales (wholegrain iron-fortified cereal compared to other interventions) ↑ *Clostridium* group XIVa (pureed beef compared to other interventions)	Not evaluated
	Iron- and zinc-fortified cereal	No changes in the Schao1 diversity index	↑*Clostridium* group XIVa ↓Bacteroidales			
	Pureed beef	No changes in the Schao1 diversity index	↑*Clostridium* group XIVa ↓*Enterobacteriaceae*			
Qasem et al. (2017)	Iron-fortified rice cereal	No changes in the Chao1 richness estimator and Shannon diversity index	No changes reported	↑Chao1 richness estimator (pureed beef compared to other interventions)	No changes between groups reported	Not evaluated
	Iron-fortified rice cereal with raspberry	↑Chao1 richness estimator, Shannon diversity index	No changes reported			
	Pureed beef	↑Chao1 richness estimator No changes in the Shannon diversity index	No changes reported			
Ordiz et al. (2020)	Common bean	No changes in Faith's Phylogenetic diversity and weighted UniFrac distances	No changes reported	No changes in Faith's Phylogenetic diversity and weighted UniFrac distances between groups	↑*Bifidobacterium* ↓*Prevotella* (cowpea compared to other intervention groups)	Not evaluated
	Cowpea	No changes in Faith's Phylogenetic diversity and weighted UniFrac distances	No changes reported			
	Corn-soy blend flour (control)	No changes in Faith's Phylogenetic diversity and weighted UniFrac distances	No changes reported			
Zambrana et al. (2019)	Rice bran (absence of intervention was used as control)	No changes reported	No changes reported	No changes in alpha diversity indices (Observed, Shannon, Inverse Simpson, and Richness) (Malian and Nicaraguan cohorts)	Malian cohort: ↑*Lactobacillus, Alloprevotella* ↓*Bifidobacteriaceae, Clostridium, Terrisoporobacter* Nicaraguan cohort: ↑*Paraprevotella, Phascolarctobacterium, Veillonella, Bifidobacterium* ↓*Lachnospiraceae, Alisonella*	Not evaluated

(*Continued*)

Table 3. Continued.

		Microbiota composition				Microbiota function
		Changes between baseline and at the end of the intervention		Changes between groups at the end of the intervention		
Reference	Complementary food	Diversity indexes	Relative abundance (order, family, or genus)	Diversity indexes	Relative abundance (order, family, or genus)	Short-chain and branched-chain fatty acids
Tang et al. (2023)	Pureed meats (beef, pork, and poultry)	↑Chao1 richness, Evenness, and Shannon Diversity estimators	↑*Blautia, Bacteroides, Ruminococcaceae, Coprococcus, Anaerostipes, Faecalibacterium, Roseburia,* Clostridiales, *Ruminococcus, Dorea, Lachnospira, Haemophilus* ↓*Escherichia, Enterobacteriaceae, Enterococcus, Klebsiella, Akkermansia*	Changes in beta diversity between groups. Increased Chao1 richness estimator for the meat group	↑*Akkermansia* (dairy compared to meat group)	No changes were observed between groups. Increased fecal butyrate for the meat group at the end of the intervention, as compared to the baseline
	Dairy products (yoghurt, cheese, and whey protein)	↑Evenness and Shannon Diversity estimators No changes in the Chao1 richness estimator	↑*Blautia, Bacteroides, Ruminococcaceae, Coprococcus, Anaerostipes, Faecalibacterium, Roseburia,* Clostridiales, *Ruminococcus, Dorea, Lachnospira, Haemophilus, Akkermansia* ↓*Escherichia, Enterobacteriaceae, Enterococcus, Klebsiella*			
Bierut et al. (2021)	Bovine colostrum and egg powder mixture	Not evaluated	Not evaluated	No changes in beta diversity between groups (weighted UniFrac distances)	Changes were reported only at the species level	Not evaluated
	Corn and soy flour (control)	Not evaluated	Not evaluated			

abundances of the genera *Paraprevotella*, *Phascolarctobacterium*, *Veillonella*, and *Bifidobacterium*, and decreased abundances of the family *Lachnospiraceae* and the genus *Allisonella* compared to the control group. Malian infants consuming rice bran had an increased abundances of the genera *Lactobacillus* and *Alloprevotella* but decreased abundances of the *Bifidobacteriaceae* family, and *Clostridium* and *Terrisoporobacte*r genera compared to the control group.

Existing evidence on the effects of plant-based foods on the fecal microbiota of weaning infants indicates no changes in microbial alpha diversity following the consumption of commercial infant cereals, legumes, or rice bran. This finding was consistent across all five trials that used plant-based foods as an intervention. Among the evaluated foods, wholegrain cereal promoted a longitudinal increase in the fecal abundance of bacteria from the order Bacteroidales in two studies. Additionally, one study reported that consumption of wholegrain cereal decreased the fecal abundance of the genus *Escherichia-Shigella,* which includes potential pathogens.

3.3. Influence of meats on the fecal microbiota of weaning infants

Three studies compared the influence of pureed meat versus other foods on the infant fecal microbiota (Krebs et al. 2013; Qasem et al. 2017; Tang et al. 2023). Krebs et al.(2013) allocated American breastfed infants to pureed beef or iron-fortified cereals during a 3-month intervention. Fruits, vegetables, teething biscuits, and unfortified cereals were allowed ad libitum, while there was no consumption of infant formula during the study. The study monitored the use of antibiotics during the trial but did not report it. No changes in the Chao1 richness index were observed between groups, nor were there longitudinal changes in the microbial richness due to pureed beef consumption. On the other hand, infants who consumed pureed beef showed an increase in the relative abundances of the group *Clostridium* XIVa and a decrease in the family *Enterobacteriaceae* compared to baseline values. Consumption of pureed beef also promoted an increased abundance of the *Clostridium* VIXa group compared to the other intervention groups.

A similar study design was used by Qasem et al.(2017) who introduced either pureed beef or iron-fortified cereals to Canadian breastfed infants for two to four weeks. During the trial, infants consumed breastmilk, but it is unknown whether they consumed infant formula or other foods. The use of antibiotics during the intervention was not reported. Unlike the findings of Krebs et al.(2013), the authors reported that infants consuming pureed beef showed a longitudinal increase in microbial alpha diversity measured by the Chao1 richness index when compared to their baseline. At the end of the study, the pureed beef intervention group also exhibited a higher Chao1 index compared to the other intervention groups. However, no changes in the relative abundance of microbial taxa were observed due to pureed beef consumption.

Tang and coauthors (2023) characterized differences in the fecal microbiota of American formula-fed infants consuming pureed meats (beef, pork, and poultry) or dairy-based foods from 5 to 12 months of age. In addition to the intervention, infants consumed infant formula, cereal, fruit, and vegetables ad libitum. There was no consumption of breastmilk. One infant in each group used antibiotics during the intervention. Of 71 infants enrolled in the trial, 64 completed it, and 57 had their microbiota composition and function assessed (27 in the meat group and 30 in the dairy group). A permutational analysis of variance indicated differences in microbial composition between intervention groups at the end of the study (p-value = 0.014). Alpha diversity indexes differed between groups at 12 months, with infants in the meat group having a higher Chao1 richness estimator (p-value = 0.002). A longitudinal increase in the Chao1 estimator was also reported for infants consuming pureed meats (Table 3).

The consumption of pureed meats drove longitudinal changes in the relative abundance of microbial taxa, with increased abundances of the genera *Bacteroides*, *Faecalibacterium*, and the family *Ruminococcaceae*, while the genus *Escherichia* and the order *Enterobacteriaceae* decreased. Only the genus *Akkermansia* differed between intervention groups, having a lower abundance at the end of the study for infants who consumed meats. The study also evaluated the concentration of SCFAs in infants' fecal samples, reporting an increase in butyrate concentration in the meat group from 5 to 12 months (1.75-fold change) but no changes between groups (Tang et al. 2023).

Current evidence suggests that meat consumption, particularly pureed beef, increases the microbial alpha diversity of the fecal microbiota in weaning infants. This result was observed in two of the three studies using pureed beef as an intervention, compared to baseline values or other interventions. Furthermore, two trials reported that pureed beef consumption increased the fecal abundance of SCFA-producing bacteria, including the group *Clostridium* XIVa and the genera *Faecalibacterium* and *Bacteroides*. In contrast, pureed beef intervention decreased the fecal abundance of the family Enterobacteriaceae in two trials.

3.4. Influence of dairy products on the fecal microbiota of weaning infants

Only two identified trials used dairy products as an intervention (Bierut et al. 2021; Tang et al. 2023). Bierut et al. (2021) assessed the influence of consuming a mixture of bovine colostrum and egg powder on the fecal microbiota of Malawian breastfed infants (9 months old). It is unknown whether infants consumed other foods, breastmilk, or infant formula during the trial. A mixture of corn and soy flour was used as a control in the 3-month intervention. Of the 277 enrolled infants, 267 completed the trial, and 263 infants had their fecal microbiota composition analyzed at the end of the intervention (12 months old). The use of antibiotics during the trial was not reported. A permutational analysis of variance reported no changes in weighted UniFrac distances between control and intervention groups (p-value = 0.374) (Table 3). Changes in microbial relative abundance were reported only at the species level. As compared to the control group, infants that consumed bovine colostrum with egg had higher abundances of *Clostridium perfringens*, *Streptococcus thermophilus*, *Megamonasrupellensis*, *Megasphaerapaucivorans*, and *Eubacterium* sp., while a lower abundance of *Lactobacillus* sp.

More changes in the fecal microbiota composition were observed in the study conducted by Tang and colleagues (2023), who assigned 5-month-old American infants, exclusively formula-fed, to dairy foods (yoghurt, cheese, and whey protein) or pureed meats. Infants consumed infant formula and other foods ad libitum but not breastmilk. One infant in each group used antibiotics during the intervention. At the end of the 7-month intervention, infants allocated to the dairy group had an increased relative abundance of the genus *Akkermansia* compared to infants who consumed pureed meats. Dairy product consumption also promoted longitudinal increases in the abundances of the genus *Bacteroides* and the order Clostridiales and decreases in the abundances of the genus *Escherichia* and the family *Enterobacteriaceae*, among other changes (Table 3). Increases in alpha diversity scores (Evenness and Shannon Diversity) were reported for the dairy group when comparing values at the baseline and the end of the intervention. On the other hand, no longitudinal changes in the concentration of fecal SCFAs and BCFAs were observed for the dairy group compared to its baseline, nor between intervention groups at the end of the study.

Limited evidence exists regarding the influence of dairy foods on the fecal microbiota of weaning infants. One study reported that a mixture of bovine colostrum and egg powder increased the fecal abundance of the genus *Eubacterium* while decreasing the abundance of *Lactobacillus* compared to the control group. Another study found that the consumption of whey, cheese, and yogurt increased the abundance of the genus *Akkermansia* compared to the control. Additionally, these dairy foods increased the fecal microbial richness compared to baseline values.

4. Discussion

This systematic review examined the effect of complementary foods on the composition and function of the fecal microbiota of infants at weaning age (4 to 12 months). Only a few clinical trials have evaluated the influence of early-life nutrition on the development of fecal microbes as infants transition from breastmilk to complementary foods. Seven studies met the inclusion criteria for this review, providing examples of the effect of plant-based foods, meats, and dairy foods on the infant fecal microbiota.

The evidence collected by this review suggests that the consumption of complementary foods by infants promotes the colonic development of microbial taxa capable of metabolizing complex carbohydrates and proteins, notably the genus *Bacteroides* (Smith and Macfarlane 1998; Martens et al. 2011). At the same time, the abundance of microbes commonly found in feces in the early months of life, such as the family *Enterobacteriaceae* and the genus *Escherichia*, decreases. This effect was observed for all the sources of foods included in this review (plant-based foods, meats, or dairy products), suggesting a natural progression in the development of the fecal microbiota as the infant ages, which is not associated with consuming a specific food item. This finding aligns with previous studies (Laursen et al. 2016; Heppner et al. 2024). However, it is important to acknowledge that the evidence collected in this systematic review lacked detailed dietary intake information regarding the intake of breastmilk, infant formula intake, and other complementary foods.

This review also suggests that plant-based complementary foods have a lesser impact on the diversity of the infant fecal microbiota compared to meats and dairy products. At the end of all five studies using plant-based foods as intervention, no changes in the fecal microbial diversity were observed between intervention and control groups. On the other hand, longitudinal increases in microbial richness were observed in two out of three trials that used pureed meats, and in one trial out of two that used dairy products. In agreement with this finding, the consumption of meats and cheeses was reported to be associated with increased fecal alpha diversity in infants, likely due to their relatively higher protein content (Laursen et al. 2016).

Commercial infant cereals were the most studied complementary plant-based foods ($n = 3$). Two studies reported that wholegrain cereals intervention during the complementary feeding period promoted the increased abundance of microbial taxa commonly found in adult feces, like the order Bacteroidales, while reducing the abundance of potential pathogens such as the genus *Escherichia-Shigella*, compared to baseline values. Similar outcomes were observed *in vitro* through the fermentation of wholegrain commercial cereals using a fecal inoculum from weaning infants, showing an increase in the abundance of the *Bacteroidaceae* family and a decrease in the abundance of the *Enterobacteriaceae* family (Gamage et al. 2017).

These changes in microbial taxa can be attributed to the high content of non-digestible carbohydrates in wholegrain cereals, particularly dietary fiber. Dietary fiber supports the development of the fecal microbiota as they reach the colon undigested and are preferentially fermented by colonic commensals producing, among other products, organic acids that ultimately impact host physiology (Yang et al. 2013; So et al. 2021). For instance, the SCFAs acetate, propionate, and butyrate have been extensively studied for their health benefits, including providing energy to colonocytes and supporting the intestinal barrier function, among others (Liu et al. 2021).

Longitudinal studies linking dietary patterns with the fecal microbiota of infants reported that fiber consumption, including the intake of infant cereals, is positively correlated with increased fecal concentrations of propionate at 6 months and alpha diversity at 12 months (Leong et al. 2018; Gómez-Martín et al. 2022). Another study positively correlated complex carbohydrate consumption with the fecal abundances of the SCFAs-producing genera *Ruminococcus* and *Lachnospira*in infants aged 6 to 12 months (Khine et al. 2020). Although there are no current recommendations for daily dietary fiber intake for infants in their first year of life, nutritional guidelines highlight the importance of consuming fiber-rich foods, such as whole grains, fruits, and vegetables, as part of a healthy diet in early infancy (Alvisi et al. 2015; U.S. Department of Agriculture and U.S. Department of Health and Human Services 2020; World Health Organization 2023).

Dietary guidelines for infants also recommend the consumption of lean meats to provide protein and micronutrients, particularly iron and zinc (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2020; World Health Organization 2023). Pureed beef was the most studied meat in the identified trials ($n = 3$). Its effect on fecal microbial diversity scores and relative abundances of microbes in weaning infants varied between studies. Nevertheless, the evidence collected in this review suggests that pureed beef beneficially increases the fecal abundance of butyrate-producers, like the genus *Bacteroides* and members of the *Clostridium* XIVa group, while reducing the abundance of the family *Enterobacteriaceae*(2 out of 3 studies). Pureed beef also increased microbial richness in infant feces in two studies, a parameter positively associated with a more stable fecal microbiota and negatively associated with biomarkers for disease development in adults (Cotillard et al. 2013; Le Chatelier et al. 2013; Tap et al. 2015).

Observed changes in the fecal microbiota composition due to pureed beef consumption are likely due to its high protein and iron content. Although the human body is efficient in digesting and absorbing protein, approximately 10% of consumed protein reaches the adult colon unabsorbed, where it is fermented by proteolytic bacteria producing SCFAs and BCFAs (Gibson, Sladen, and Dawson 1976; Macfarlane, Cummings, and Allison 1986; Macfarlane et al. 1992). Longitudinal trials positively correlated animal protein consumption with increased fecal abundance of the *Bacteroides* genus and alpha diversity in weaning infants (Laursen et al. 2016; Khine et al. 2020; Gómez-Martín et al. 2022). In contrast, iron supplementation was associated with increased fecal abundance of the genera *Escherichia/Shigella* and *Clostridium* in weaning infants (Jaeggi et al. 2015). *In vitro* fecal fermentation studies using adult inoculum demonstrated that consuming beef promotes the development of taxa from the genera *Clostridium* and *Peptoclostridium,* leading to the production of acetate, propionate, butyrate, isobutyrate, and isovalerate (Shen, Chen, and Tuohy 2010; Xiao et al. 2021).

Neither of the two studies evaluating fecal metabolites reported changes in the concentration of BCFAs due to the intervention of complementary foods. Currently, the effect of BCFAs on host physiology is not well-characterized.

These organic acids are considered markers of protein fermentation (Macfarlane et al. 1992), which in turn also produces potentially deleterious metabolites like ammonia and p-cresol(Richardson, McKain, and John Wallace 2013; Saito et al. 2018). For instance, ammonia is neurotoxic when present in high levels in the blood while high doses of p-cresol are toxic to colonic epithelial cells (Rangroo Thrane et al. 2013; Andriamihaja et al. 2015). In this context, moderation is key. Evidence in adults has shown that excessive consumption of animal protein, concomitant with insufficient fiber intake, disrupts the fecal microbiota (Russell et al. 2011), but when consumed as part of a balanced diet, it beneficially increases the colonic microbial diversity (Trefflich et al. 2021).

Longitudinal trials in weaning infants observed that dairy consumption was negatively correlated with the fecal abundances of the *Bacteroides* genus and the *Enterobacteriaceae* family, and cheese intake was associated with increased microbial alpha diversity (Laursen et al. 2016; Smith-Brown et al. 2019; Khine et al. 2020; Gómez-Martín et al. 2022). In adults, a trial comparing high versus low consumption of milk, cheese, and yoghurt reported increased relative abundance of the species *Streptococcus thermophilus* for participants consuming higher amounts of dairy foods (Swarte et al. 2020). However, no changes in fecal microbiota diversity were observed between groups (Swarte et al. 2020). This result aligns with the findings of Bierut et al.(2021) who observed no changes in microbial diversity but the increased abundance of *S. thermophilus* in weaning infants consuming a mixture of bovine colostrum and egg powder.

A recent systematic review of the impact of dairy products on the human fecal microbiota reported no changes in the microbial diversity in response to dairy interventions but increased abundances of the *Lactobacillus* and *Bifidobacterium* genera in three out of seven studies included (Aslam et al. 2020). These genera include members having benefits on children's gastrointestinal health, suggesting that dairy consumption positively impacts the fecal microbiota of weaning infants (Szajewska and Hojsak 2020). The effect of dairy products on colonic microbes, compared to other complementary foods, may be explained by the presence of lactose, which fosters the growth of lactic acid bacteria and lactate production, contributing to SCFAs production through cross-feeding interactions (Van den Abbeele et al. 2021). Whey protein also promotes the growth of the *Bifidobacterium* and *Lactobacillus* genera, stimulating SCFAs production (Sánchez-Moya et al. 2017).

In addition, fermented dairy products, like cheese and yoghurt, are also a source of living microorganisms and microbial metabolites, the consumption of which may support a balanced fecal microbiota (Okoniewski et al. 2023). Ultimately, changes in the fecal microbiota promoted by dairy products may benefit host health, as their consumption has been linked to a reduced risk of developing chronic diseases, such as type two diabetes and obesity, as reported in meta-analyses involving children and adults (Tong et al. 2011; Lu et al. 2016). Dairy foods (rather than whole milk as a drink) are recommended for weaning infants in the first year of life as part of a nutritionally balanced diet, providing protein, vitamins, and minerals, particularly calcium and riboflavin (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2020; World Health Organization 2023).

This systematic review focused on the impact of complementary foods on the fecal microbiota of infants aged 4 to 12 months. Although often understudied in microbiota investigations, the weaning period plays a crucial role in the maturation of the fecal microbiota. The description of the evidence collected here sheds light on how a few complementary foods introduced to infants at the early stages of weaning affect their fecal microbiota, contributing to filling a current knowledge gap in infant nutrition. Another strength of this study was the inclusion of randomized controlled trials, which are the gold standard methodology for assessing outcomes of dietary interventions.

It is important to emphasize that although this review concentrated on the effects of individual complementary foods, introducing weaning infants to a diversified complementary diet is crucial for meeting their nutritional requirements and supporting the adequate maturation of their fecal microbiota. As recommended by dietary guidelines, diversity is a key factor in healthy complementary feeding patterns (Alvisi et al. 2015; U.S. Department of Agriculture and U.S. Department of Health and Human Services 2020; World Health Organization 2023). Infants consuming a diverse diet are less likely to develop allergies and atopic diseases (Lv et al. 2024). On the other hand, infants who lack a varied diet at weaning are more prone to nutrient deficiency, ultimately compromising their health status later in life (Arimond and Ruel 2004; Victora et al. 2008). In regards to the fecal microbiota, longitudinal investigations showed that increased dietary diversity during weaning is associated with higher microbial diversity and richness, contributing to stabilize the microbiota (Laursen et al. 2016; Homann et al. 2021).

One limitation of this review is that only seven trials satisfied the eligibility criteria. This was due to the scarcity of interventions assessing the impact of complementary foods on the fecal microbiota of weaning infants, highlighting the need for more clinical research in this area. However, a search strategy limited to only papers published in English excluded potentially eligible records in other languages, which may have contributed to the small number of included studies. Due to the lack of available data for the same food intervention, meta-analyses of the effect of complementary foods on the microbiota of weaning infants could not be performed. Instead, a narrative discussion of the results of the included trials was performed. The limited data on the same food interventions also compromised the synthesis capacity of this systematic review, highlighting the urgent need for more randomized controlled trials to evaluate the relationship between complementary foods and the fecal microbiota of weaning infants.

One study reported considerable antibiotic usage during the trial, with 52 out of 95 infants receiving antibiotics (Zambrana et al. 2019). Additionally, three studies did not provide information about antibiotic usage during the intervention period (Krebs et al. 2013; Qasem et al. 2017;

Bierut et al. 2021). Antibiotics are known to alter the fecal microbiota composition, potentially confounding the effects of the dietary intervention (Bokulich et al. 2016). One trial included in this review had an intervention time lower than 1 month and may not have been long enough to capture persistent changes in the fecal microbiota (Qasem et al. 2017). Although there are no absolute guidelines about the duration of food intervention trials in microbiome research, evidence in adults suggests that short-term dietary interventions lead to rapid but temporary modifications in the fecal microbiota (David et al. 2014; Swann et al. 2020).

Most of the identified trials (4 out of 7) compared the effect of one intervention against another in the absence of a relatively inert food used as a control (Krebs et al. 2013; Qasem et al. 2017; Plaza-Diaz et al. 2021; Tang et al. 2023). This approach makes it difficult to isolate the individual impact of each food on the fecal microbiota and may lead to confounding results and limited conclusions. One study analyzed the fecal microbiota of a small subset of 14 participants from a total of 45 enrolled infants, reducing its statistical power and potentially leading to false conclusions (Krebs et al. 2013). Additionally, three trials characterized the fecal microbiota of weaning infants as a secondary outcome, and not analyzing fecal samples from all infants or collection time points (Krebs et al. 2013; Bierut et al. 2021; Plaza-Diaz et al. 2021).

Infants under 6 months of age were enrolled in four trials (Krebs et al. 2013; Qasem et al. 2017; Plaza-Diaz et al. 2021; Tang et al. 2023), including infants at 4 months (Qasem et al. 2017; Plaza-Diaz et al. 2021). This contrasts with current complementary feeding recommendations, which recommend exclusive breastfeeding for around the first 6 months (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2020; World Health Organization 2023). Although this recommendation is flexible and can be adapted to each infant's needs, the fecal microbiota develops rapidly during the first year of life, and even a difference of a few months in age can lead to distinct compositions among infants (Stewart et al. 2018; Oyedemi et al. 2022; Heppner et al. 2024). Nevertheless, parents may introduce their infants to complementary foods earlier than recommended. As observed by the Feeding Infants and Toddlers Study 2016, the largest dietary intake survey in infants and toddlers performed in the USA, only 15% of infants aged 4–5.9 months were exclusively breastfed (Roess et al. 2018). Furthermore, the introduction of solids between 4 and 6 months is recommended for infants at risk of developing food allergies (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2020). Given this scenario, this review included trials involving infants aged at least 4 months. This is justified because it is likely that infants will be introduced to complementary foods at around 4 months of age.

Importantly, Krebs et al. (2013) and Qasem et al. (2017) reported contrasting effects on the fecal microbiota of infants for the same food intervention, either pureed beef or infant cereals. Regarding the effect of the pureed beef intervention, Krebs and coauthors (2013) observed changes in the abundance of microbial taxa but no changes in microbial diversity, while the latter noticed alterations in the microbial richness but not in the relative abundance of microbes (Qasem et al. 2017). These contrasting results may be due to other key factors influencing the fecal microbiota in early postnatal life, such as mode of delivery, type of feeding (breastmilk versus infant formula), maternal diet, and geographical location (Rutayisire et al. 2016; Taylor et al. 2023). The influence of these factors on colonic microbes reduces as the infant ages but is still evident in the early stages of weaning (Fallani et al. 2011; Sillner et al. 2021).

In addition, several other host factors affecting the fecal microbiota, such as the circadian cycle and intestinal transit time, were not considered in the selected studies (Liang, Bushman, and FitzGerald 2015; Müller et al. 2020; Heppner et al. 2024). Notably, while all studies included in this review assessed the dietary intake of participants, it is not evident whether changes in dietary intake over time, particularly for breastmilk or infant formula consumption, were accounted for in the microbiota analyses. This limitation is especially relevant for infant formula, as its consumption may increase during weaning as a replacement for breastmilk.

In the example above, Krebs and coauthors (2013) recruited only vaginally delivered American infants, while Qasem and colleagues (2017) recruited Canadian infants delivered vaginally, by cesarean, and through other modes. It is worth noticing that the trials included in this review were conducted in different parts of the world and that the geographical location of the infants will have influenced the fecal microbiota composition, as evidenced in infants and adults (Fallani et al. 2011; Suzuki and Worobey 2014). The fecal microbiota also varies between individuals and within the same individual over time, potentially reacting differently to the same dietary intervention for each person (Salonen et al. 2014; Olsson et al. 2022). Furthermore, infants normally consume complementary foods alongside other foods, often in mixed meals, which may influence the digestion and absorption of nutrients and subsequent microbiota response.

In addition, methods to characterize fecal microbes were heterogeneous among studies, limiting the comparison of the results. All trials used 16S rRNA sequencing but amplified different hypervariable regions, leading to potential disagreements in microbial taxa identification and resolution (Kameoka et al. 2021). Different bioinformatic pipelines and reference databases were used, which are likely to affect the taxonomic assignment and the estimation of the relative abundance and diversity scores of the microbial community (Marizzoni et al. 2020; Ramakodi 2022). Identified trials used fecal samples to assess the colonic microbiota due to the ease of collection, transportation, and storage, and its noninvasive and cost-effective nature. However, fecal samples mainly reflect the microbial composition of the distal colon, failing to fully represent microbial communities free in the colonic lumen or attached to the colonic mucosa and other parts of the colon (Flynn et al. 2018).

Only one trial evaluated the influence of complementary foods on the production of both SCFAs and BCFAs by the fecal microbiota of infants (Tang et al. 2023). The lack of metabolite production analyses in most of the included studies is a crucial limitation of the existing data thatraises questions about the potential impact of observed changes in taxonomic composition on the overall functionality of the microbial community. It is important to note that evaluating the impacts of dietary interventions on the fecal microbiota requires more than just analyzing microbial composition. Ultimately, understanding the functional aspects of the microbiota is more relevant for host health. Despite interindividual variations in the microbial composition, the fecal microbiota of healthy individuals has similar functions (Huttenhower et al. 2012). On the other hand, imbalances in the production of microbial metabolites are associated with colonic dysbiosis and increased risk of disease (Arrieta et al. 2015; Zuo et al. 2019; Chen et al. 2022). Further research, particularly studies using a multi-omics approach to assess both the impact of foods on microbial composition and function, is necessary to better understand how introducing complementary foods affects the fecal microbiota in infants.

In conclusion, this systematic review included seven food intervention trials that assessed the influence of plant-based foods, meats, or dairy products on the fecal microbiota of weaning infants. The evidence collected suggests a natural progression in the increasing abundance of fecal microbes capable of metabolizing complex carbohydrates and proteins as infants age, which does not seem to be related to the consumption of any specific complementary food. Two studies evaluated wholegrain cereals as an intervention, whereas three other trials investigated the effects of pureed beef. These foods increased the fecal abundance of microbes producing SCFAs compared to baseline values, with this effect observed in two trials for each food. Additionally, pureed beef increased fecal microbial richness in two studies. However, the conclusions of this review are limited by the small number of included studies and their varying methodologies and reported outcomes. Further research assessing the impact of complementary foods on both the composition and function of the fecal microbiota in weaning infants is essential to fill this knowledge gap in infant nutrition.

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Author contributions

VGS contributed to the search and screening of studies, data analysis and interpretation, and writing of the manuscript. JNT contributed to the search and screening of studies, and data analysis and interpretation. All authors contributed to the design of this systematic review and to the text and the content of the manuscript, including revisions and edits. All authors approve the content of the manuscript and agree to be held accountable for the work.

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