



Breastfeeding Affects Concentration of Faecal Short Chain Fatty Acids During the First Year of Life: Results of the Systematic Review and Meta-Analysis

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Short chain fatty acids (SCFAs) are important metabolites of the gut microbiota. It has been shown that the microbiota and its metabolic activity in children are highly influenced by the type of diet and age. Our aim was to analyse the concentration of fecal SCFAs over two years of life and to evaluate the influence of feeding method on the content of these compounds in feces. We searched PubMed/MEDLINE/Embase/Ebsco/Cinahl/Web of Science from the database inception to 02/23/2021 without language restriction for observational studies that included an analysis of the concentration of fecal SCFAs in healthy children up to 3 years of age. The primary outcome measures—mean concentrations—were calculated. We performed a random-effects meta-analysis of outcomes for which ≥ 2 studies provided data. A subgroup analysis was related to the type of feeding (breast milk vs. formula vs. mixed feeding) and the time of analysis (time after birth). The initial search yielded 536 hits. We reviewed 79 full-text articles and finally included 41 studies ($n = 2,457$ SCFA analyses) in the meta-analysis. We found that concentrations of propionate and butyrate differed significantly in breastfed infants with respect to time after birth. In infants artificially fed up to 1 month of age, the concentration of propionic acid, butyric acid, and all other SCFAs is higher, and acetic acid is lower. At 1–3 months of age, a higher concentration of only propionic acid was observed. At the age of 3–6 months, artificial feeding leads to a higher concentration of butyric acid and the sum of SCFAs. We concluded that the type of feeding influences the content of SCFAs in feces in the first months of life. However, there is a need for long-term evaluation of the impact of the observed differences on health later in life and for standardization of analytical methods and procedures for the study of SCFAs in young children. These data will be of great help to other researchers in analyzing the relationships between fecal SCFAs and various physiologic and pathologic conditions in early life and possibly their impact on health in adulthood.

Keywords: microbiota, metabolome, gut, infant, feeding

INTRODUCTION

During the first 2–3 years of life, the gut microbiome undergoes rapid and important changes in bacterial community structure and function (1, 2). This maturation phase is characterized by an early abundance of *Bifidobacterium*, *Bacteroides*, and *Escherichia*, which are gradually replaced by obligate anaerobic bacteria, particularly members of the Firmicutes phylum, such as *Clostridiaceae* and *Lachnospiraceae* (3). One of the most important factors in the composition of the gut of infants is human milk in the diet of infants (1, 2). Consumption of breast milk is associated with increased abundance of *Bifidobacteria* and decreased abundance of Firmicute *Lachnospiraceae* (4, 5). Formula-fed infants tend to be richer in *Bacteroides*, *Escherichia*, *Enterobacteriaceae*, *Clostridium* (1, 6) and other bacteria associated with a more mature microbiota (7). Some evidence exists that bacterial colonization of the gut by breast milk bacteria is dose-dependent. Pannaraj et al. found that infants whose diet consisted of 75% human milk (>) obtained about 27% of their gut bacteria from breast milk, whereas infants who were less breastfed obtained only about 17% of their bacteria from milk—this difference in bacterial uptake decreases as infants get older and are exposed to other bacterial sources (8). Infant gut colonization is also influenced by other factors, including cesarean delivery (9) antibiotic use (10) and maternal body mass index (BMI) (11). Like formula feeding, these factors are associated with early intestinal maturation (i.e., higher numbers of Firmicutes), which may have adverse effects on immune system development (12, 13) and may increase the risk of obesity in children (11, 14).

Microorganisms are linked to diet and various physiological situations through their ability to produce gut microbial metabolites. Since humans lack the enzymes to break down indigestible carbohydrates, these carbohydrates are fermented by bacterial species in the gastrointestinal tract in the cecum and colon (15). This phenomenon produces various metabolites such as short-chain fatty acids (SCFAs) as the main group (16). In bacteria themselves, SCFAs are a waste product essential for the formation of the redox equivalent in the anaerobic situation (17). These metabolites are organic acids with one to six carbon atoms, including acetate (C2), propionate (C3), and butyrate (C4) (18). SCFAs have various and crucial activities in humans; in this regard, butyrate is probably the best known SCFA for health and disease (19). Butyrate is the most important energy pool for colonocytes. This metabolite plays an influential role in cancer control by stimulating cell apoptosis and modulating gene expression *via* histone deacetylases (HDACs) (20). In addition, propionate is also an energy pool for many cells, such as epithelial cells, and could be transferred to the liver where it is involved in gluconeogenesis (19). Lastly, another SCFA, acetate, could enter peripheral tissues and be involved in lipogenesis and cholesterol metabolism. Recent *in vivo* documents show that it also plays a crucial role in central appetite modulation (21). On the other hand, SCFAs have been shown to influence bacterial gene expression. In this context, acetate induces gene expression in *Salmonella typhimurium*, for example, which occurs through bacterial invasion due to the formation of acetyl phosphate (22).

In addition, exposure of enterohaemorrhagic *Escherichia coli* (EHEC) to butyrate has been found to induce gene expression involved in adherence to epithelial cells *via* recognition of Lrp, the leucine-responsive regulatory protein (23).

Creating gut microbiota occurs primarily in the period of infancy. *Bifidobacterium* dominates during breastfeeding whilst in case of formula feeding *Bacteroides* and *Clostridioides* abundance is higher. A milestone in creating the microbiome is the introduction of solid foods, usually around 6 months of age. Consistently, over time and depending on the infant's diet, there are differences in the concentration of short-chain fatty acids (SCFAs)—the main metabolites of the microbiota. It has been shown that infants who were exclusively breastfed had lower levels of total SCFA: propionate, butyrate, iso butyrate, valerate and isovalerate, and higher levels of intermediate metabolites such as lactate and succinate. In addition, a higher relative acetic acid content was observed. This information is based on single observational studies and no systematic review with meta-analysis has been performed so far. Therefore, we decided to perform a systematic review and meta-analysis of the concentration of SCFAs (propionic, butyric, acetic) in the first 3 years of life and analyse the changes in these values over time and regarding the diet type (breastfeeding vs. formula). These data will be of great help to other researchers in analyzing the relationships of fecal SCFAs with various physiological and pathological conditions at early in life and potentially their effects on health in adulthood. They will also be helpful in comparing the effects of dietary interventions, new formula composition, the use of pre- and probiotics, and drugs on the fecal concentration of SCFAs.

METHODS

Search Strategy and Inclusion Criteria

We commissioned three independent authors (LS, MFT, PT) to search PubMed/MEDLINE/Embase/Ebsco/Cinahl/Web of Science from database inception to 23/02/2021 without language restriction for observational studies involving analysis of SCFA levels in healthy children up to 3 years of age. We also included randomized controlled trials (RCTs) that compared a specific intervention with placebo/no intervention, the latter group being of interest.

The following search terms were used.

String for Pub Med./Cinahl/Web Of Science: (newborn OR child, newborn OR full term infant OR human neonate OR human newborn OR infant, newborn OR neonate OR neonatus OR newborn OR newborn baby OR newborn child OR newborn infant OR newly born baby OR newly born child OR newly born infant OR child OR child OR children) AND (feces OR fecal excretion OR feces OR fecal excretion OR feces OR stool OR stools) AND (short chain fatty acid OR fatty acid, short chain OR short chain fatty acid OR acetic acid OR acetate OR acetate salt OR acetate sodium OR acetic acid OR sodium acetate OR butyric acid OR butanoate sodium OR butanoic acid OR butyrate OR butyrate sodium OR butyric acid OR n butyrate OR n butyric acid OR sodium butyrate OR sodium n butyrate OR propionic acid OR propionate OR propionic

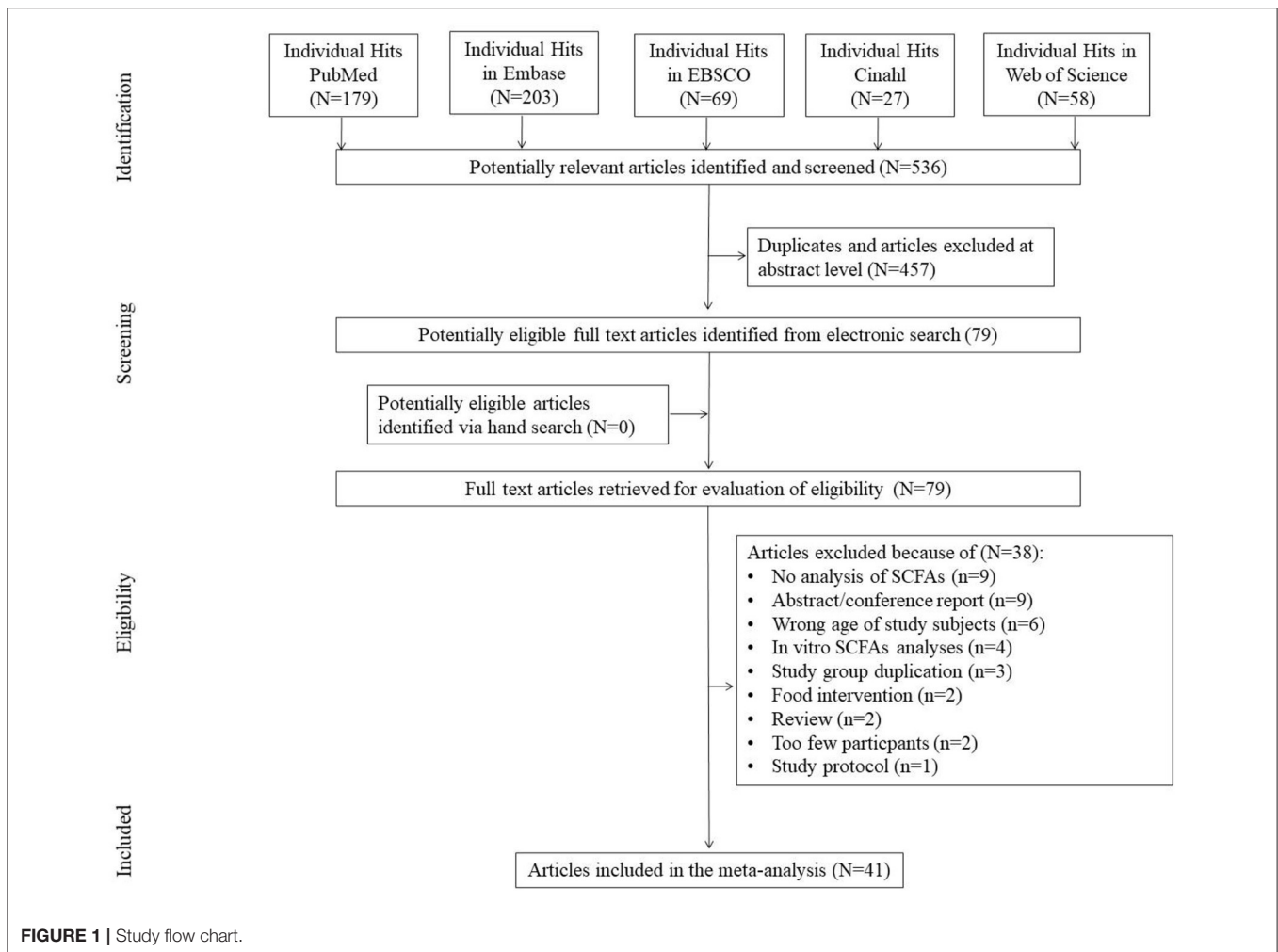


FIGURE 1 | Study flow chart.

acid) AND (observational study OR non experimental studies OR non experimental study OR nonexperimental studies OR nonexperimental study OR observation studies OR observation study OR observational studies OR observational study OR observational study as topic OR observational studies as topic OR cross-sectional study OR cross-sectional design OR cross-sectional research OR cross-sectional studies OR cross-sectional study OR cohort analysis OR analysis, cohort OR cohort analysis OR cohort life cycle OR cohort studies OR cohort study OR randomized controlled trial OR controlled trial, randomized OR randomized controlled study OR randomized controlled trial OR randomized controlled study OR randomized controlled trial OR trial, randomized controlled).

In PubMed: filter: *HUMANS*

String for Embase: ('newborn'/exp OR 'child, newborn' OR 'full term infant' OR 'human neonate' OR 'human newborn' OR 'infant, newborn' OR 'neonate' OR 'neonatus' OR 'newborn' OR 'newborn baby' OR 'newborn child' OR 'newborn infant' OR 'newly born baby' OR 'newly born child' OR 'newly born infant' OR 'child'/exp OR 'child' OR 'children') AND ('feces'/exp OR 'fecal excretion' OR

'faeces' OR 'fecal excretion' OR 'feces' OR 'stool' OR 'stools') AND ('short chain fatty acid'/exp OR 'fatty acid, short chain' OR 'short chain fatty acid' OR 'acetic acid'/exp OR 'acetate' OR 'acetate salt' OR 'acetate sodium' OR 'acetic acid' OR 'sodium acetate' OR 'butyric acid'/exp OR 'butanoate sodium' OR 'butanoic acid' OR 'butyrate' OR 'butyrate sodium' OR 'butyric acid' OR 'n butyrate' OR 'n butyric acid' OR 'sodium butyrate' OR 'sodium n butyrate' OR 'propionic acid'/exp OR 'propionate' OR 'propionic acid') AND ('observational study'/exp OR 'non experimental studies' OR 'non experimental study' OR 'nonexperimental studies' OR 'nonexperimental study' OR 'observation studies' OR 'observation study' OR 'observational studies' OR 'observational study' OR 'observational study as topic' OR 'observational studies as topic' OR 'cross-sectional study'/exp OR 'cross-sectional design' OR 'cross-sectional research' OR 'cross-sectional studies' OR 'cross-sectional study' OR 'cohort analysis'/exp OR 'analysis, cohort' OR 'cohort analysis' OR 'cohort life cycle' OR 'cohort studies' OR 'cohort study' OR 'randomized controlled trial'/exp OR 'controlled trial, randomized' OR 'randomized controlled study' OR 'randomized controlled trial' OR 'randomized

TABLE 1 | Study groups characteristics.

ID	References/ type of study	Aim	Specification of healthy controls	N of subjects/ N whole study group/ visits in case of multiple analyses.	T/PT: HBD (mean ± SD), weeks.	Delivery type N (cesarean/ vaginal)	Nutrition method in infancy	
1	Ben et al. (29)/case control	Effect of GOS on intestinal microbiota.	Receiving formula	24/164. Visit at age: 3 m.	T	ND	MF:45	
1a			Receiving human milk.	17/164. Visit at age: 3 m.	T		BF:24	
2	Ben et al. (30)/case control	The effect of infant formula milk consisting of GOS on intestinal microbiota and the fermentation characteristics in term infants in comparison with that of human milk	Receiving breastfeeding.	26/271. Visit at age: 3 m, 6 m.	ND	ND	BF:26	
2a			Receiving formula.	52/271. Visit at age: 3 m, 6 m.	ND		10/13	MF:52
2b								MF:52
2c								MF:52
3	Berni Canani et al. (48)/cross-sectional	To evaluate gut microbiota composition and butyrate production in children affected by non-IgE-mediated cow's milk allergy.	Healthy children at the mean age 12.9 ± 7.4 months	23/69	ND	10/13	BF (for at least 1 mth):14	
4	Brink et al. (36)/cohort	To investigate fecal microbiota and metabolites at different ages in infants who were breastfed (BF), received cow milk—based formula (MF), or received soy-based formula (SF) or no longer breastfeeding (NLB).	Receiving breastfeeding	Visit at age 3 m: 16/42	T	ND	BF:16	
4a			Visit at age 6 m: 20/54	T	BF:20			
4b			Visit at age 9 m: 12/35	T	BF:35			
4c			Visit at age 12 m:14/53	T	BF:14			
4d			Receiving milk formula	Visit at age 3 m: 12/42	T		MF:12	
4e			Visit at age 6 m: 19/54	T	MF:19			
4f			Visit at age 9 m: 11/35	T	MF:11			
4g			Visit at age 12 m: 14/53	T	MF:14			
4h			Receiving soya based formula	Visit at age 3 m: 14/42	T		MF:14	
4i			Visit at age 6 m: 15/54	T	MF:15			
4j			Visit at age 9 m: 12/35	T	MF:12			
4k	Visit at age 12 m: 15/53	T	MF:15					
4l	Switched to milk formula when breastfeeding was not possible.	Visit at age 12 m:10/53	T	MF:10				
5	Bazanella et al. (52)/RCT	To determine the effects of a bifidobacteria-containing formula on the healthy human intestinal microbiome during the first year of life	Receiving placebo formula	49/106 Visits at age: 1, 3, 5, 7, 9, 12 month.	T	27/22	MF:11; BF:38.	
5a			Receiving breastfeeding	9/106 Visits at age: 1, 3, 5, 7, 9, 12 month.	T		6/3	BF:9.
5b								
5c								
5d								
5e								
5a								
5b								
5c								

(Continued)

TABLE 1 | Continued

ID	References/ type of study	Aim	Specification of healthy controls	N of subjects/ N whole study group/ visits in case of multiple analyses.	T/PT: HBD (mean \pm SD), weeks.	Delivery type N (cesarean/ vaginal)	Nutrition method in infancy
5d							
5e							
6	Bakker-Zierikzee et al. (31)/case control	To compare the effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable <i>Bifidobacterium animalis</i> on the composition and metabolic activity of the intestinal microbiota.	Receiving breast milk	Visit at age 5 days: lactate 43/120, SCFA 32/120. Visit at age 10 days: lactate 44/120, SCFA 33/120. Visit at age 4 weeks: lactate 35/120, SCFA 28/120. Visit at age 8 weeks: lactate 31/120, SCFA 22/120. Visit at age 12 weeks: lactate 26/120, SCFA 24/120. Visit at age 16 weeks: lactate 22/120, SCFA 17/120.	ND	Total: 4/59	BM:63
6a							
6b							
6c							
6d							
6e							
6f			Receiving standard formula.	Visit at age 5 days: lactate 13/120, SCFA 12/120. Visit at age 10 days: lactate 19/120, SCFA 15/120. Visit at age 4 weeks: lactate 16/120, SCFA 13/120. Visit at age 8 weeks: lactate 15/120, SCFA 14/120. Visit at age 12 weeks: lactate 15/120, SCFA 14/120. Visit at age 16 weeks: lactate 14/120, SCFA 12/120.		Total: 5/14	Total: MF:19
6g							
6h							
6i							
6j							
6k							
7	Díaz et al. (37)/cohort	To establish potential links between type of formula substitutes, microbiota and desensitization in infants with Non-IgE mediated cow's milk protein allergy.	10 age-matched healthy infants (12–24 months old), with a normal diet consuming cow's milk	10/27	ND	ND	MF
8	Differding et al. (38)/cohort	To find a link between complementary feeding and infant gut microbiota composition, diversity and SCFAs.	Early introduction to complementary foods.	Visit at age 3 month: 18/67 Visit at age 12 month: 13/67	T	Total: 11/7	BF ever:14
8a							
8b			Late introduction to complementary foods.	Visit at age 3 month: 47/67 Visit at age 12 month:36/47	T	Total: 33/16	BF ever:39
8c							

(Continued)

TABLE 1 | Continued

ID	References/ type of study	Aim	Specification of healthy controls	N of subjects/ N whole study group/ visits in case of multiple analyses.	T/PT: HBD (mean ± SD), weeks.	Delivery type N (cesarean/ vaginal)	Nutrition method in infancy
9	Edwards et al. (32)/case control	To compare of SCFAs concentration at 2 and 4 weeks of age in babies fed exclusively breast milk or infant formula	Receiving exclusively breastfeeding	Visit at age 2 weeks: 19/47	T	ND	BF:19
9a				Visit at age 4 weeks: 12/47			BF:12
9b			Receiving formula.	Visit at age 2 weeks: 28/47			MF:28
9c				Visit at age 4 weeks: 27/47			MF:27
10	Fleming et al. (53)/RCT	To investigate whether <i>B. breve</i> BBG-001 affected: intestinal permeability; intestinal microbiota composition and SCFAs synthesis	Receiving placebo.	16/29	ND	ND	ND
11	Heath et al. (33)/case control	To assess whether SCFAs content is linked with sleep in infants.	Receiving standard care.	29/57	T	ND	ND
11a			Feeding themselves from weaning.	29/57			
12	Holscher et al. (54)/RCT	To compare the proportions of fecal bifidobacteria in infants fed formula with or without prebiotics for 6 weeks.	Receiving breastfeeding.	33/105 Visits at age: 0, 3, 6 weeks.	T	ND	BF:33
12a							
12b							
12c			Receiving control formula.	33/105 Visits at age: 0, 3, 6 weeks.			MF:33
12d							
12e							
13	Iszatt et al. (39)/cohort	To investigate whether exposure to toxicants affect SCFAs and gut microbial composition	Norwegian Microbiota Cohort (NoMIC). Not specified.	70/608	PT (n = 13) and T (n = 57)	13/55	BF:59
14	Kien et al. (40)/case control	To evaluate process of colonic fermentation of carbohydrate to SCFAs	Infants born at 28–32 weeks' gestation and had reached 2–4 weeks' postnatal age receiving formulas: GP (carbohydrate source, 50% lactose-50% glucose polymer) and LAC (containing all the carbohydrate as lactose).	7/15 (LAC)	PT: 28–32	ND	F:7
14a				8/15 (GP)			F:8
15	Kim et al. (55)/RCT	To the potential role for probiotics in the prevention of childhood eczema.	Receiving placebo, no sign of eczema.	7/33	T	0/7	BF:3, MF:3, M:1
16	Knol et al. (56)/RCT	To assess GOS and fructooligosaccharides (FOS) impact on microbiota structure and function.	Receiving breastfeeding.	19/68	T	4/15	BF:11
16a			Receiving standard formula.	19/68		2/16	BF:19
17	Kok et al. (34)/case control	To observe the impact of extensively hydrolyzed (EH) proteins or free amino acids (AA) on intestinal microbiota composition and immune reactivity.	Healthy term infants from birth to 60 days of age	17/75 Visits at age: baseline, 30, 60 day.	T	ND	BF:17
17a							
17b							
18	Kosuwon et al. (57)/RCT	To determine the effect of consuming Young Child Formula (YCF) supplemented with short chain galactooligosaccharides and long chain fructooligosaccharides (scGOS/lcFOS, ratio 9:1) and <i>B. breve</i> M-16V on the development of the fecal microbiota in healthy toddlers.	Healthy toddlers	64/129	T	26/38	MF: 64

(Continued)

TABLE 1 | Continued

ID	References/ type of study	Aim	Specification of healthy controls	N of subjects/ N whole study group/ visits in case of multiple analyses.	T/PT: HBD (mean ± SD), weeks.	Delivery type N (cesarean/ vaginal)	Nutrition method in infancy	
19	Liu et al. (58)/RCT	To compare the effects of human milk and infant formulas on fecal SCFAs and bacterial composition in human infants.	Receiving breastfeeding.	35/40/120	T	Total: 0/74	BF:35	
19a	Lund-Blix et al. (41)/Cohort Dahl et al. (27)/Cohort ^a	Investigation of the association between gestational age and gut microbiome.	Receiving standard formula.	37/40/120	PT (n = 15), T (n = 59)	Vaginally born only.	MF:37	
20			No antibiotics intake, Norwegian Microbiota Study (NoMIC) cohort	Visit at age 10 day: 74/519			BF: 74	
20a				Visit at age 4 month: 54/519			PT (n = 6), T (n = 48)	BF: 54
20b				Visit at age 12 month: 91/519			PT (n = 20), T (n = 71)	Not BF (formula and solid food): 71
21	Maldonado et al. (59)/RCT	To evaluate the safety of a follow-on formula with <i>Lactobacillus salivarius</i> CECT5713 in 6 month old children.	Receiving formula.	40/80 Visits at age: baseline, 3, 6 month.	T	ND	MF:40	
21a	Mentula et al. (60)/RCT	To find differences in the fecal microbial composition and metabolic function and assess the probiotic efficacy in colicky infants.	Healthy term infants at the age 1–6 weeks	9/18	T	3/6	BF:8; MF:1.	
21b								
22								
23	Midtvedt et al. (49)/Cross-sectional	To observe the development of functionally active intestinal microbiota.	Receiving exclusively breastfeeding up to 6 month of age.	1/17 Visits at age: baseline, 30, 60 day	T	ND	BF:11	
23a			Receiving exclusively breastfeeding up to 3 month of age.	8/17 Visits at age: baseline, 30, 60 day				
23b			Receiving exclusively breastfeeding up to 1 month of age.	2/17 Visits at age: baseline, 30, 60 day				
23c			Receiving formula	6/17				
24	Mohan et al. (61)/RCT	To evaluate the effect of <i>Bifidobacterium lactis</i> Bb12 supplementation on body weight, gut fermentation patterns, and immunologic parameters in preterm infants.	Receiving placebo.	32/69 Visits at age: 1, 2, 3 week.	PT:31.27±2.56	29/3	MF:6 MF:5; HM+HMF: 27.	
24a	Nilsen et al. (42)/cohort	To identify the nutrient utilization and SCFAs production in infants during the first year of life	Not specified.	Visits at age 0: 100/100	T	22/78	BF (3–6 months): 83; BF (6–9 months): 73; BF (9–12 months): 49.	
24b								
25								
25a	Nocerino et al. (62)/RCT	To assess the efficacy of <i>Bifidobacterium animalis subsp. lactis</i> BB-12 [®] in colicky infants	Exclusively breastfeeding, aged ≤ 7 weeks.	Visits at age 3 month: 100/100	T	19/21	BF: 40	
25b				Visits at age 6 month: 100/100				
25c				Visits at age 12 month:99/100				
26				40/80 Visits: enrolment, 28 day of the study.				
26a								

(Continued)

TABLE 1 | Continued

ID	References/ type of study	Aim	Specification of healthy controls	N of subjects/ N whole study group/ visits in case of multiple analyses.	T/PT: HBD (mean ± SD), weeks.	Delivery type N (cesarean/ vaginal)	Nutrition method in infancy
27	Nogacka et al. (43)/cohort	To evaluate the impact of intrapartum antimicrobial prophylaxis (IAP) on microbiome	IAP +	18/40 Visits at age: 2, 10, 30, and 90 days.	T	0/40	BF:11; MF:7
27a							
27b			IAP –	22/40 Visits at age: 2, 10, 30, and 90 days.			BF:18; MF:4
27c							
28	Oshiro et al. (63)/RCT	To evaluate the efficacy of Bifidobacterium breve (BBG-01) along with mother's colostrum and breast milk on growth and fecal parameters in preterm infants	Infants born between 24 and 31 weeks of gestation and with body weights < 1,500. g	Visit at age 0 week: 15/35	PT:28.2±3.3	15/3	BM: 13; M: 4
28a				Visit at age 1 week: 14/35			
28b				Visit at age 2 weeks: 17/35			
28c				Visit at age 4 weeks: 18/35			
28d				Visit at age 8 weeks: 17/35			
29	Park et al. (44)/cohort	To evaluate the effect of composition and function of gut microbiota at 6 months on atopic dermatitis up to 24 months in early childhood.	Not specified.	84/132	T	25/58	BF:25 MF:10 M:47
30	Pourcyrous et al. (50)/Cross-sectional	Analysis of interactions among GI tract, microbiota, diet and risk of NEC.	Delivery at <32 weeks of gestation. Expressed maternal breast milk. Preterm formula.	13/32	PT:27.3±2.3	11/2	BM+HMF: 13
30a				19/32	PT:26.6±2.1	10/19	MF:19
31	Quin et al. (35)/case control	Determination if probiotics intake affects infant microbiome, immune markers, SCFA production and health.	Healthy term infants, predominantly breastfed, mother and child without probiotic treatment	52/87	T	15/36	BF: 52
32	Rao et al. (45)/cohort	Comparison of stool microbiota and SCFAs of term infants with congenital gastrointestinal surgical conditions (CGISCs) to healthy control.	Not specified.	Visit at age 1 week: 23/73;	T	12/24	BF: 26
32a				Visit at age 2 week: 17/73.			
33	Roduit et al. (46)/cohort	Analysis of association between fecal SCFA and development of allergic diseases and atopic sensitization later in life.	Children from the Protection against Allergy—Study in Rural Environments (PASTURE).	301	ND	38/263	At 2 months: BF: 184; MF: 45; M: 50.
34	Sierra et al. (64)/RCT	To check the prebiotic effect of GOS on intestinal microbiota to decrease infections and allergy manifestations in healthy infants during the first year of life	Gestational age of 37–42 weeks, birth weight > 2,500 g and were exclusively formula fed for at least 15 days prior to enrolment.	Total: 37/365 Visit at enrolment: 37/365; Visit at age 4 month: 29/365.	T	55/122 (all study group)	MF: 177 (all study group)
34a							
35	Stansbridge et al. (65)/RCT	To assess the efficacy of <i>Lactobacillus rhamnosus</i> GG to modify enteric carbohydrate fermentation in preterm infants.	Preterm < 33 gestational weeks	10/20 SCFAs were measured in samples pooled in the following age ranges (days): 1–7; 8–14; 15–21; 22–28; 1–28.	PT: <33	ND	3–10 day of life: BM: 9; MF: 1. Later: all MF.
35a							
35b							
35c							

(Continued)

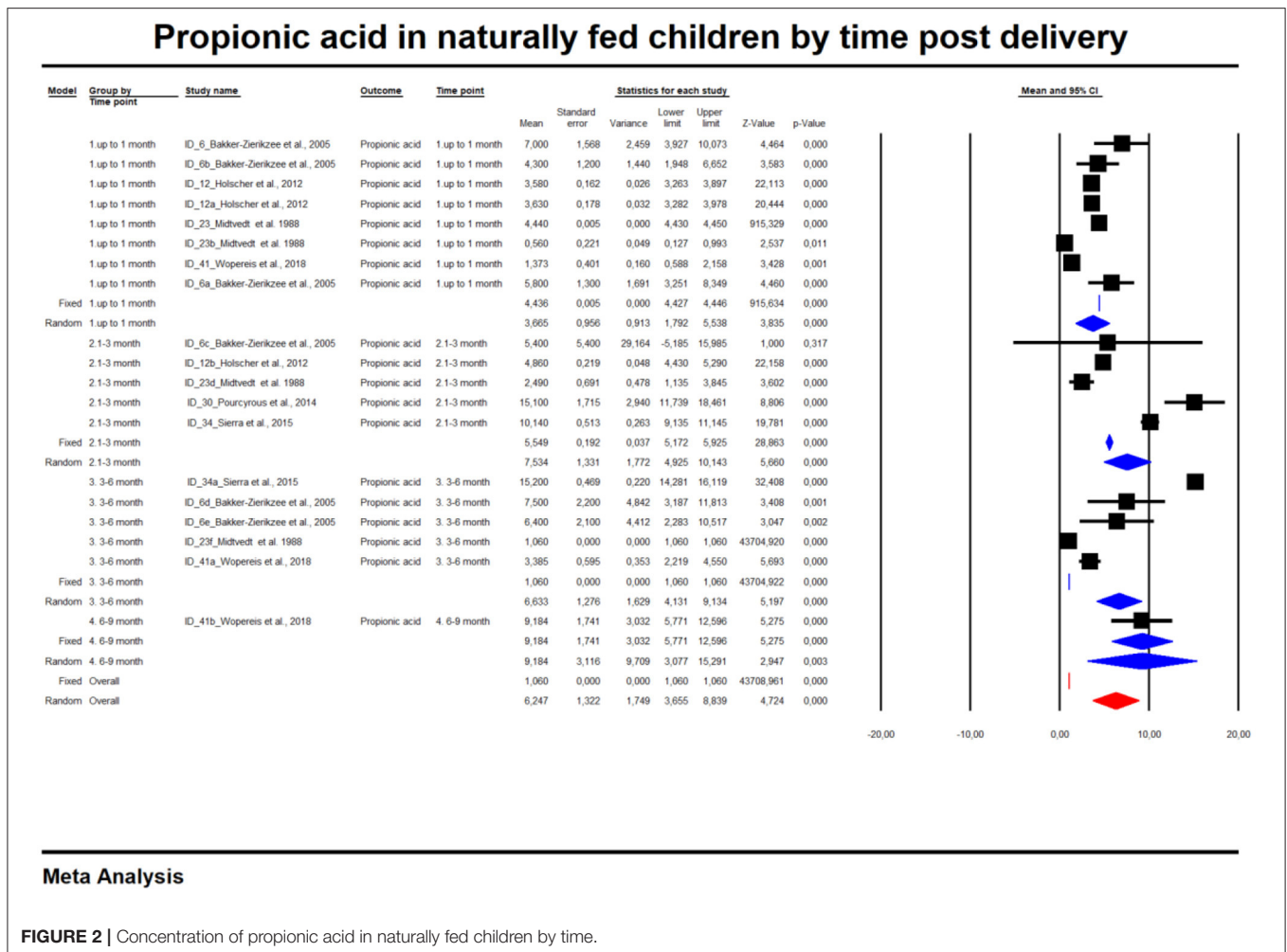
TABLE 1 | Continued

ID	References/ type of study	Aim	Specification of healthy controls	N of subjects/ N whole study group/ visits in case of multiple analyses.	T/PT: HBD (mean ± SD), weeks.	Delivery type N (cesarean/ vaginal)	Nutrition method in infancy
35d 36	Ta et al. (47)/cohort	To evaluate the functionality of the gut microbiome and metabolome in eczema.	Not specified.	Visit at age 3 weeks: 13/63;	T	10//20	BF: 2; MF: 16; M: 12.
36a 36b 36c				Visit at age 3 months: 16/63; Visit at age 6 months: 27/63; Visit at age 12 months: 26/63.			
37	Tauchi et al. (51)/cross-sectional	To elucidate the dynamics and equilibria of the developing microbiota in preterms.	Not specified.	59 Samples were collected at 5 days and approximately 1 month post-delivery.	T (n = 42) and PT (n = 17) : 34.2 ± 2.7	33/26	Term: BF: 18; M: 22; F: 2 PreTerm: M: 16, BM: 1.
37a 38	Underwood et al. (66)/RCT	To compare the effect of two prebiotic/probiotic products on weight gain, stool microbiota, and stool SCFA of premature infants.	Birth weight 750–2,000 g, gestational age < 35 weeks	29/90	PT:29.3 ± 2.6	23/6	BM: 11; FM: 7; M: 11.
39	Westerbeek et al. (67)/RCT ^a	To determine the effects of enteral supplementation of a prebiotic mixture of neutral and acidic oligosaccharides (scGOS/lcFOS/pAOS) on the fecal microbiota and microenvironment in preterm infants.	Gestational age < 32 weeks and/or birth weight < 1,500 g,	58/113 Samples collected at: <48 h; days: 7, 14, 30.	PT: 31.1 ± 6.4	26/32	BM: 33; FM: 25.
39a 39b 39c							
40	Wopereis et al. (69)/RCT	Analysis of the efficacy of amino-acid based formula (AAF) including specific synbiotics on oral and gastrointestinal microbiota of infants with non-IgE mediated cow's milk allergy (CMA).	Receiving formula. Receiving breastfeeding	Visit at week 0: 33/122	T	ND	BF: 51; FM: 36.
40a 40b 40c				Visit at week 8 (controls): 31/122 Visit at week 8 (healthy subjects): 48/122 Visit at week 12: 26/122 Visit at week 26: 23/122.			
41	Wopereis et al. (68)/RCT	The effects of interventions and breast-feeding on fecal microbiota were investigated and linked with eczema	Receiving formula. Receiving breastfeeding	57/138 Visits at age: 4, 12, 26 weeks.	ND	11/46	MF:57
41a 41b				30/138 Visits at age: 4, 12 weeks, 51/138 Visits at age: 4, 12 weeks,		5/25 10/41	BF:30 MF (partially hydrolyzed): 51

N, number; GOS, galacto-oligosaccharide; ME, median; IQR, interquartile range; IgE, immunoglobulin E; NEC, necrotizing enterocolitis; GI, gastrointestinal; T, term; PT, pre-term; BF, breast fed; BM, Breast Mother Milk; MF, milk formula; SF, soya based formula; HM, Human milk; M, mixed fed; HM + HMF, Human Milk and Human Milk Fortifier.

^asource of data indicated by Lund-Blix et al. (41).

^bsource of data (28).



Meta Analysis

FIGURE 2 | Concentration of propionic acid in naturally fed children by time.

controlled study’ OR ‘randomized controlled trial’ OR ‘trial, randomized controlled’).

We supplemented the electronic search with a manual review of reference lists of relevant meta-analyses and reviews.

The inclusion criteria were:

- (i) data on the concentration of SCFAs in stool,
- (ii) age 0–3 years.

We excluded reviews, systematic reviews, editorials, single case reports, opinion articles and editorials/perspectives.

Data Abstraction

The protocol for this systematic review, meta-analysis and meta-regression was registered in the International Prospective Register of Systematic Reviews (Prospero database registration number CRD42022313244). Data on the study design and study group of each study were extracted by two independent reviewers (MFT, LS) in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) standard. If data were missing for the review, the authors were contacted twice by email at 2-week intervals to obtain additional

information. Disagreements were resolved by consensus, involving a clinical lead (BŁ).

Outcomes

Co-primary endpoints were the concentrations of: (i) acetic acid, (ii) propionic acid, (iii) butyric acid and (iv) all SCFAs. Secondary outcomes were concentrations of other SCFAs, like valeric, isobutyric, caproic, and others.

Data Synthesis and Statistical Analysis

We conducted a random effects (24) meta-analysis of outcomes for which ≥3 studies contributed data, using Comprehensive Meta-Analysis V3 (<http://www.meta-analysis.com>). We explored study heterogeneity using the chi-square test of homogeneity, with *p* < 0.05 indicating significant heterogeneity. All analyses were two-tailed with alpha = 0.05.

Group differences in continuous outcomes were analyzed as the pooled means. A subgroup analyses regarding feeding type (breast vs. formula vs. mixed) and time of analysis (time post delivery) were conducted. We utilized the following time ranges: up to ≤1 month, >1–≤3 months, >3–≤6 months, >6–≤9 months, >9–≤12 months and >12 months of age. Categorical

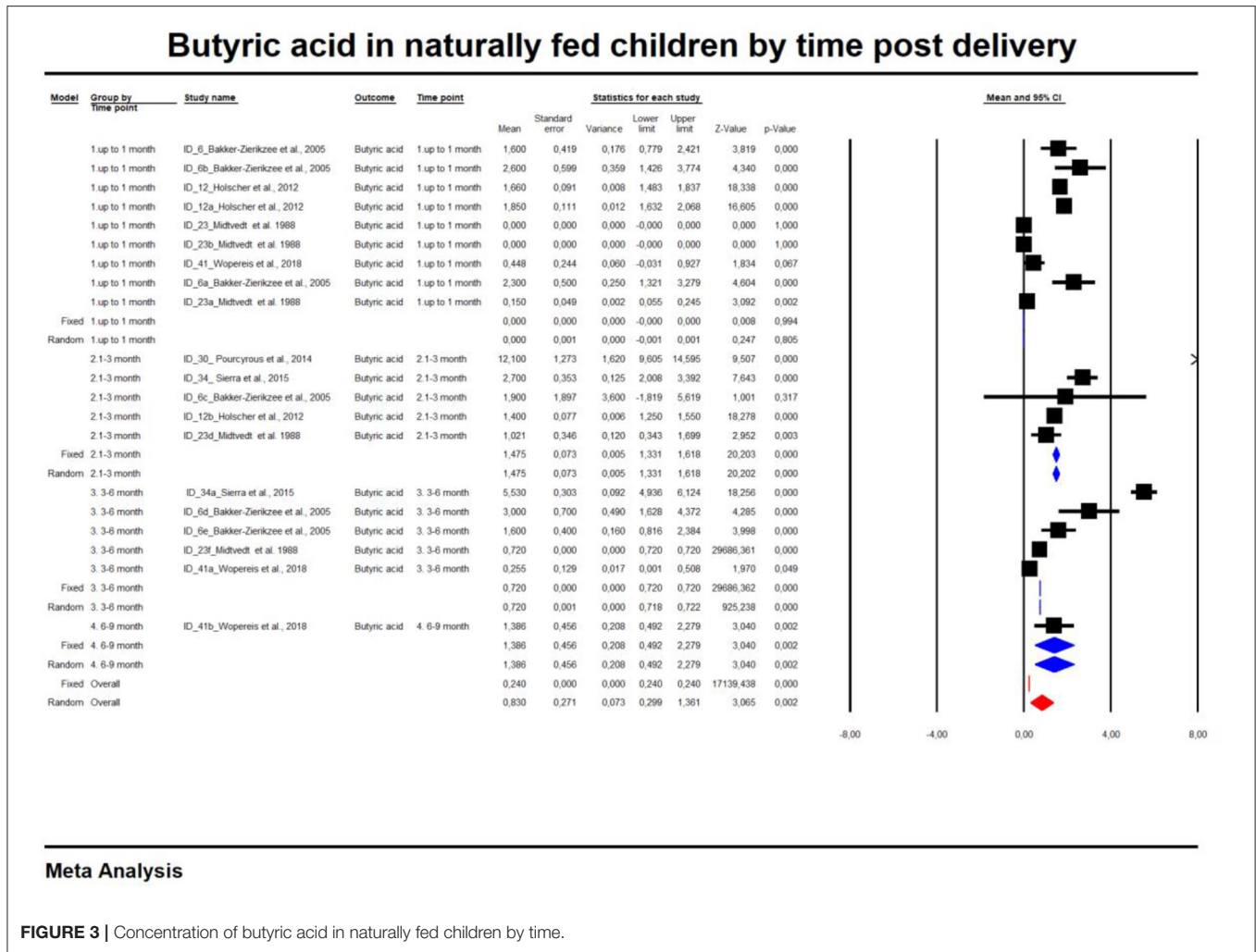


FIGURE 3 | Concentration of butyric acid in naturally fed children by time.

outcomes were not analyzed. Finally, we inspected funnel plots and used Egger’s regression test (25) and the Duval and Tweedie’s trim and fill method (26) to quantify whether publication bias could have influenced the results.

Risk of Bias

As our systematic review and meta-analysis contains single arms form RCTs predominantly (involving healthy children only, receiving placebos and also data abstracted from control groups (healthy children) in case control-studies, we decided not to evaluate the bias, as such score refers to a whole study, not its part.

RESULTS

Search Results

The first search yielded 536 hits. Four hundred fifty-seven studies were excluded because they were duplicates and/or because they were assessed at the title/abstract level. No additional articles were identified via the hand search. Two studies that qualified for meta-analysis included data from sources indicated by the authors of the publication (27, 28). Subsequently, all

79 full-text articles were reviewed. Of these, 38 were excluded because they did not meet the inclusion criteria. The reasons for exclusion were: no analysis of SCFAs/no data available (N = 9), abstract/conference report (N = 9), wrong age of subjects studied (N = 6), *in vitro* analyses (N = 4), duplicate study group (N = 3), food intervention (N = 2), review (N = 2), too few participants (N = 2), study protocol (N = 1) (Figure 1), leaving 41 studies included in the meta-analysis.

Characteristics of the Studies and the People Studied

A total of 41 studies were included with a total number of n = 2,457 SCFAs analyses. We pooled data on controls from case-control (29–35), cohort (36–47) and cross-sectional studies (48–51). Data on children from RCTs control groups were also included (52–69). There were both term and pre-term delivered children (but only healthy) included. Other data, delivery type and nutrition method are placed in Table 1. Please note that the subgroups in the first column reflect the data provided by the authors according to the time of the analyses, which correspond to the data in the forest plots.

SCFA Concentrations by Time of Analysis (Time After Delivery)

For the meta-analysis, we used data from studies that had only means and standard deviations and where standardization of concentration units was possible. Consequently, pooling was only possible for data obtained up to 9 months of age. The raw data with all abstracted information on concentrations at 3 years of age can be found in the **Supplementary Tables 1–4**.

For the sake of clarity, we performed a subgroup analysis by time of evaluation separately for breastfed and formula-fed children. We found that the concentrations of C2, C3, C4 and pooled SCFAs in artificially fed children did not differ by time of evaluation ($p > 0.05$). In contrast, the concentrations of propionate and butyrate in breastfed infants differ significantly with respect to the time period after birth (**Figures 2, 3**). **Table 2** also details the concentrations (mean, standard deviation and ranges) of C2, C3, and C4 at different life stages in relation to feeding method.

Overall, an Egger test does not indicate publication bias in all but three cases (**Figure 4**) (Egger test: $p > 0.05$). However, the Duval and Tweedie method adjusted values in only one case; 1 study to the left of the mean was truncated for propionic acid in formula-fed infants (point estimate of random model: 4.8622; 95% CI: 4.7881–4.9364, Q value = 2,049.520).

In the final part of our study, we analyzed data on SCFAs other than those previously described. The results are presented in **Table 3**.

SCFA Concentrations by Feeding Time

In the last part of our analyses, we decided to analyse how diet was related to the levels of SCFAs in specific age groups. In this case, pooling synthesis was possible due to data availability as well as the units to be standardized for children up to 6 months of age. We found that in the vast majority of infants, formula feeding significantly increased the level of SCFAs. In infants up to 1 month of age who are artificially fed, the concentration of propionic acid, butyric acid and all SCFAs is higher and that of acetic acid is lower. At 1–3 months of age, a higher concentration of only propionic acid was observed. At 3–6 months of age, artificial feeding leads to a higher concentration of butyric acid and the sum of SCFAs. The results are shown in **Table 4** and **Figures 5, 6**. In one case—up to 1 month, butyric acid—we found that Egger's test indicated publication bias. Using the method of Duval and Tweedie, 8 studies were trimmed to the left of the mean, with a point estimate of the random-effects model: 0.028; 95% CI: 0.009–0.046, Q value = 3,373.605.

DISCUSSION

This paper is the systematic review aimed at analyzing SCFAs over 2 years of life and the influence of feeding method on the content of these compounds in feces. However, based on the available data, only the concentration of fecal SCFAs in healthy children in the first year of life was meta-analyzed. We also presented detailed data on the concentration of SCFAs in feces by life span of the newborn (up to 1 month, 3–6 months

and 6–9 months old), which can serve as a reference for other researchers. We have shown that breastfeeding is associated with changes in propionate and butyrate concentrations in the first 9 months of life and that this effect is not observed in children fed with artificial infant formula. On the other hand, a higher concentration of SCFAs (propionic acid, butyric acid, all SCFAs) in the stool is observed in artificially fed children compared to breastfed children at different stages of life. Only in the first month of life is the concentration of acetic acid lower in artificially fed children.

The content of SCFAs in the stool is the result of a trade-off between the production of these compounds by the intestinal bacteria, their uptake and their consumption *in situ*, in the gastrointestinal tract. Due to the numerous important metabolic functions performed by SCFAs during development and the impossibility (for ethical reasons) of determining their concentration in the blood of healthy newborns, the concentration of SCFAs in stool is an indirect marker of their content in the body. This meta-analysis includes a comparative evaluation of the effects of breastfeeding on the concentration of SCFAs in the stool of children up to 9 months of age, i.e., during a period when solid food is either not introduced into the diet or only introduced to a small extent.

The meta-analysis compared the effect of diet on the stool content of the three most important SCFAs, namely acetic acid, propionic acid and butyric acid, with the sum of SCFAs. The content of other acids could not be included in the meta-analysis because there was insufficient data for such a synthesis. The observed changes in the concentration of SCFAs in feces could be related to changes in the composition and function of the microbiota during the first phase of life. Acetic acid is the most abundant SCFA in the gut, its content being twice that of propionic and butyric acids (70, 71). It is produced by most of the anaerobic bacteria inhabiting the gut; for example, *Bacteroides* spp. and *Akkermansia muciniphila* produce acetic and propionic acids (71, 72). Bacteria of the genus *Clostridioides* produce three important SCFAs (73, 74) of which butyric acid is most important for maintaining a healthy gut. Organisms such as *Anaerostipes*, *Clostridioides*, *Coprococcus*, *Dorea*, *Eubacterium*, *Faecalibacterium*, *Roseburia* and *Ruminococcus* produce butyric acid, the latter three being the most abundant in the gut (75, 76). *Eubacterium* and *Anaerostipes* interact with *Bifidobacterium* to increase their ability to produce butyric acid. *Bifidobacterium* is involved in the production of butyric acid by producing acetic acid through the fermentation of carbohydrates. Subsequently, the acetic acid is converted to butyric acid by *Eubacterium* and *Anaerostipes* through a process known as cross-feeding (77). These properties of infant bacteria, *B. bifidum* and *B. breve*, are taken over by adult *Bifidobacterium* strains and by the end of the second year of life the pattern of adult SCFA production is achieved (16). This confirms the importance of this early period in shaping the metabolism of these compounds. Furthermore, *B. bifidum* is able to produce acetic acid by fermenting mucins (78). Only *A. muciniphila* and *Verrucomicrobium* have similar properties (79).

The data we have presented confirm that changes in the composition of the microbiota in the first phase of life can

TABLE 2 | The mean concentrations of SCFAs (μmol/g) by nutrition method and time range after birth.

	Time point	Number of studies	Effect size (mean)	SE	Variance	Lower limit	Upper limit	Test Z (z value)	Test Z (p-value)	Q value	df (Q)	p-value	heterogeneity (I ² from fixed effect analysis)
Formula-fed	Acetic acid												
	Up to 1 month	7	50.45	9.12	83.23	32.56	68.33	5.53	0.00	1,791.94	6.00	0.00	99.67
	1–3 months	5	54.37	10.86	117.91	33.09	75.65	5.01	0.00	382.12	4.00	0.00	98.95
	3–6 months	2	62.91	17.29	299.11	29.01	96.81	3.64	0.00	3.60	1.00	0.06	72.21
	Total between									0.41	2.00	0.81	
	Overall	14	53.59	6.48	41.95	40.89	66.28	8.27	0.00	2,449.03	13.00	0.00	99.47
	Propionic acid												
	Up to 1 month	7	12.23	2.33	5.41	7.67	16.79	5.26	0.00	1,639.5478	6.00	0.00	99.63
	1–3 months	5	13.97	2.78	7.73	8.52	19.42	5.03	0.00	31.6183312	4.00	0.00	87.35
	3–6 months	2	15.83	4.52	20.39	6.98	24.68	3.50	0.00	4.70742213	1.00	0.03	78.76
	Total between									0.58	2.00	0.75	
	Overall	14	13.34	1.66	2.75	10.08	16.59	8.04	0.00	2,049.4582	13.00	0.00	99.37
	Butyric acid												
	Up to 1 month	6	3.30	1.02	1.03	1.30	5.29	3.24	0.00	662.404243	5.00	0.00	99.25
	1–3 months	5	5.16	1.16	1.34	2.90	7.43	4.47	0.00	10.1095259	4.00	0.04	60.43
3–6 months	2	5.48	1.87	3.50	1.81	9.14	2.93	0.00	2.85E-02	1.00	0.87	0.00	
Total between									1.93	2.00	0.38		
Overall	13	4.40	0.89	0.79	2.66	6.14	4.96	0.00	934.66	12.00	0.00	98.72	
All SCFAs (not specified)													
Up to 1 month	5	55.92	4.84	23.41	46.44	65.40	11.56	0.00	42.54	4.00	0.00	90.60	
1–3 months	4	62.10	5.92	35.10	50.49	73.71	10.48	0.00	11.52	3.00	0.01	73.96	
3–6 months	1	68.60	16.47	271.26	36.32	100.88	4.17	0.00	0.00	0.00	1.00	0.00	
total between									1.02	2.00	0.60		
Overall	10	58.91	3.68	13.56	51.69	66.13	16.00	0.00	55.97	9.00	0.00	83.92	
Breast fed	Acetic acid												
	Up to 1 month	8	50.04	9.13	83.40	32.14	67.94	5.48	0.00	4,345.05	7.00	0.00	99.84
	1–3 months	5	52.26	11.62	135.01	29.49	75.03	4.50	0.00	2,740.10	4.00	0.00	99.85
	3–6 months	5	67.16	11.53	132.85	44.57	89.75	5.83	0.00	1,968.54	4.00	0.00	99.80
	6–9 months	1	59.50	25.99	675.52	8.56	110.44	2.29	0.02	0.00	0.00	1.00	0.00
	Total between									1.48	3.00	0.69	
	Overall	19	55.88	6.56	43.00	43.03	68.73	8.52	0.00	14,431.66	18.00	0.00	99.88
	Propionic acid												
	Up to 1 month	8	3.66	0.96	0.91	1.79	5.54	3.83	0.00	420.24	7.00	0.00	98.33
	1–3 months	5	7.53	1.33	1.77	4.93	10.14	5.66	0.00	140.69	4.00	0.00	97.16
	3–6 months	5	6.63	1.28	1.63	4.13	9.13	5.20	0.00	939.09	4.00	0.00	99.57
	6–9 months	1	9.18	3.12	9.71	3.07	15.29	2.95	0.00	0.00	0.00	1.00	0.00
	Total between									8.19	3.00	0.04	
	Overall	19	6.25	1.32	1.75	3.65	8.84	4.72	0.00				100.00
	Butyric acid												
Up to 1 month	9	0.01	0.01	0.00	0.00	0.02	2.42	0.02	679.59	8.00	0.00	98.82	
1–3 months	5	1.48	0.07	0.01	1.33	1.62	20.12	0.00	84.44	4.00	0.00	95.26	
3–6 months	5	0.72	0.01	0.00	0.71	0.74	93.00	0.00	280.73	4.00	0.00	98.58	
6–9 months	1	1.39	0.46	0.21	0.50	2.28	3.04	0.00	0.00	0.00	1.00	0.00	
Total between									5,869.80	3.00	0.00		
Overall	20	0.84	0.27	0.07	0.30	1.37	3.08	0.00	5,876,411.12	19.00	0.00	100.00	
All SCFAs (not specified)													
Up to 1 month	5	45.28	6.86	47.02	31.84	58.72	6.60	0.00	63.13	4.00	0.00	93.66	
1–3 months	3	71.30	9.22	85.06	53.22	89.38	7.73	0.00	113.98	2.00	0.00	98.25	
3–6 months	2	59.83	11.33	128.32	37.63	82.03	5.28	0.00	0.02	1.00	0.89	0.00	
Total between									5.30	2.00	0.07		
Overall	10	57.98	9.23	85.23	39.89	76.08	6.28	0.00	220.12	9.00	0.00	95.91	

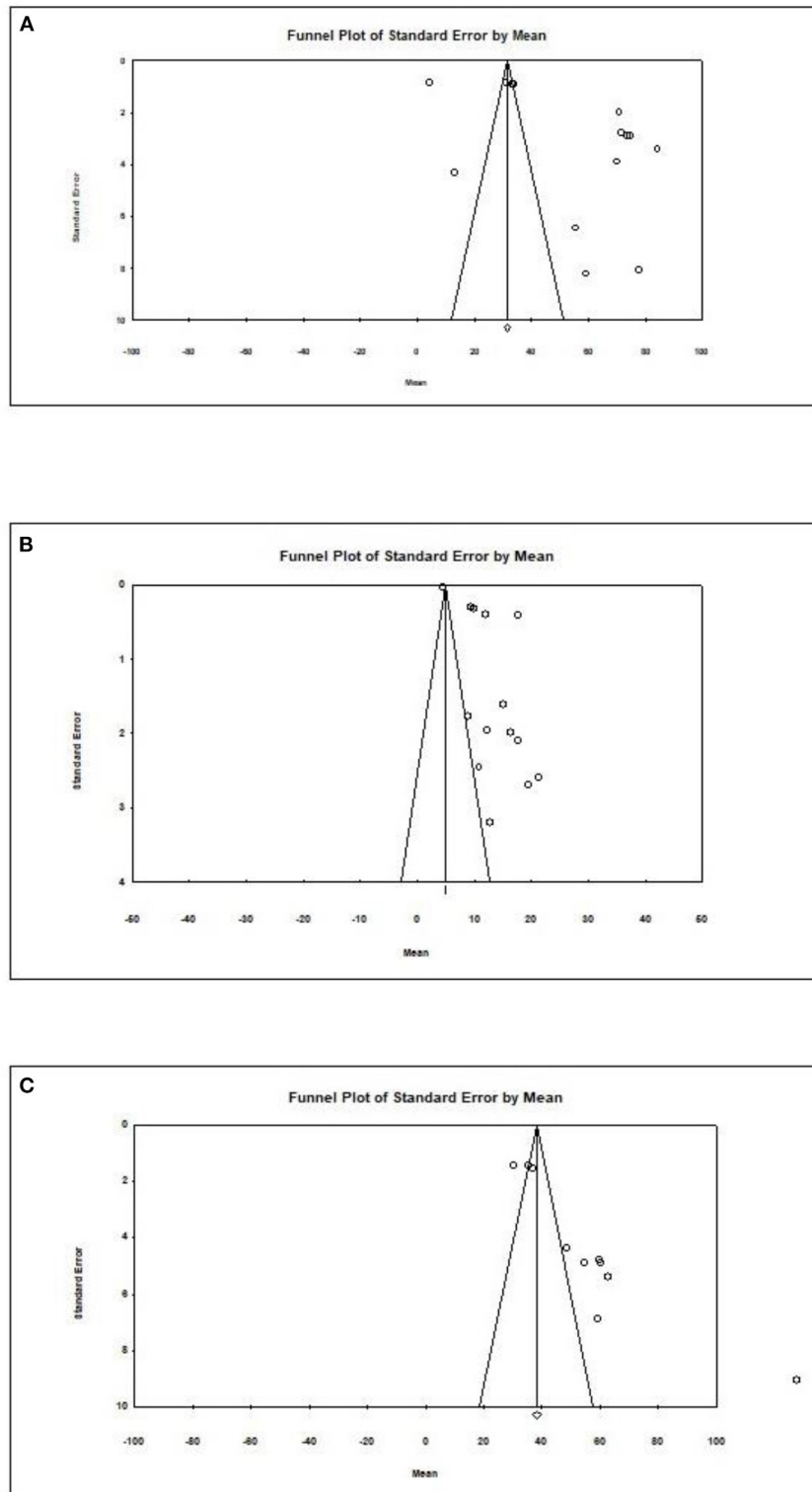


FIGURE 4 | Publication bias for different outcomes in present metaanalysis. **(A)** Acetic acid/Formula. **(B)** Propionic acid/Formula. **(C)** All SCFAs/Breast.

TABLE 3 | The concentrations of other SCFAs ($\mu\text{mol/g}$) in healthy children by time.

References	Study ID	Time range	Time when evaluated	Time unit when evaluate day (day/week/month)	Other SCFA name	Concentration (Mean)	Concentration (SD)	Concentration unit
Bakker-Zierikzee et al. (31)	6		5	Day	iC4-5 SCFA. sum of isobutyrate. isovalerate and valerate;	2	2.37	$\mu\text{mol/g}$
	6a	UP TO 1 MONTH	10	Day		2.6	2.3	
	6b		4	Week		2.1	2.11	
	6f		5	Day		1.1	1.39	
	6g		10	Day		3.2	12.39	
	6h		4	Week		5.5	9	
Edwards et al. (32)	9		2	Week	Isovaleric acid	*M 2.1	*R 0–153.0	$\mu\text{mol/g}$
	9a		2	week		*M 6.1	*R 0–13.8	
	9b		4	Week		*M 2.1	*R 0–3.5	
	9c		4	Week		*M 6.3	*R 0–17.7	
Fleming et al. (53)	10		21	Day	Lactic acid	*M 4.6	IQR 3.65–5.12	mmol/g
Holscher et al. (54)	12		0	Week	Valeric acid	2.06	1.74	$\mu\text{mol/g}$
	12a		3	Week		1.41	1.37	
	12c		0	Week		2.38	1.91	
	12d		3	Week		1.46	1.13	
Iszatt et al. (39)	13		1	Month	Isovaleric acid	0.28	0.41	$\mu\text{mol/g}$
Kien et al. (40)	14 (I)		2–4	Week	Isobutyric acid	0.01	0.016	kcal/day
	14 (II)		2–4	Week	Isovaleric acid	0.03	0.017	
	14 (III)		2–5	Week	Valeric acid	0	0.007	
Nilsen et al. (42)	25		0	Month	All other (isobutyrate. isovalerate. valerate)	9.8	nd	%
Lund-Blix et al. (41)	20 (I)		10	Days	Isobutyric acid	PT: *M 0.0 T:*M 0.0	PT: *R 0.0–0.07 T:*R 0.0–0.14	$\mu\text{mol/g}$
	20 (II)		10	Days	Isovaleric acid	PT: *M 0.1 T:*M 0.04	PT: *R 0.0–0.26 T:*R 0.0–0.12	
	20 (III)		10	Days	Valeric acid	PT: *M 0.0 T:*M 0.0	PT: *R 0.0–0.08 T:*R 0.0–0.04	
	20 (IV)		10	Days	Isocaproic acid	PT: *M 0.0 T:*M 0.0	PT: *R 0.0–0.0 T:*R 0.0–0.0	
	20 (V)		10	days	Caproic acid	PT: *M 0.0 T:*M 0.0	PT: *R 0.0–0.0 T:*R 0.0–0.1	
Wopereis et al. (68)	41 (I)		4	Week	Valeric acid	0.0086	2.79397E-10	$\mu\text{mol/g}$
	41 (II)		4	Week	Isobutyric acid	0.0086	2.79397E-10	
	41 (III)		4	Week	Isovaleric acid	0.0086	2.79397E-10	
Brink et al. (36)	4		3	Month	Valeric acid	10.8 (*M. positive mode)	2.08 (IQR)	log transformed peak areas
	4d		3	Month		13.2(*M. positive mode)	4.18 (IQR)	
	4h	1–3 MONTHS	3	Month		(*M. positive mode)	13.1 (IQR)	
Bakker-Zierikzee et al. (31)	6c		8	Week	iC4-5 SCFA. sum of isobutyrate. isovalerate and valerate;	1.6	7.5	$\mu\text{mol/g}$
	6i		8	Week		2.9	2.62	

(Continued)

TABLE 3 | Continued

References	Study ID	Time range	Time when evaluated	Time unit when evaluate day (day/week/month)	Other SCFA name	Concentration (Mean)	Concentration (SD)	Concentration unit
Differding et al. (38)	8		3	Month	Other SCFA Name (*Low abundance SCFAs including isobutyric acid, valeric acid, isovaleric acid, heptanoic acid, and hexanoic acid have been grouped into the "other" category for visual clarity)	4.4	nd	%
	8b		3	Month		3.9	nd	
Holscher et al. (54)	12b		6	Week	Valeric acid	0.97	0.87	μmol/g
	12e		6	Week		1.23	0.98	
Nilsen et al. (42)	23a		3	Month	All other (isobutyrate, isovalerate, valerate)	5.6	nd	%
Pourcyrous et al. (50)	30, 30a		first 80 days of life	Day	Isobutyric acid	N: 20.82 A:10.6	N:25.2 A:23.5	μmol/g
Brink et al. (36)	4a		6	Month	Valeric acid	10.5 (*M. positive mode)	2.14 (IQR)	log transformed peak areas
	4e		6	Month		15.3 (*M. positive mode)	2.21 (IQR)	
	4i		6	Month		13.2 (*M. positive mode)	2.4 (IQR)	
Bakker-Zierikzee et al. (31)	6d	3-6 MONTHS	12	Week	iC4-5 SCFA, sum of isobutyrate, isovalerate and valerate;	3.5	3.9	μmol/g
	6j		12	Week		3.2	1.87	
	6e		16	Week		2.2	2.06	
	6k		16	Week		4.9	2.77	
Nilsen et al. (42)	23b		6	Month	All other (isobutyrate, isovalerate, valerate)	1.7	nd	%
Lund-Blix et al. (41)	20a (I)		4	Month	Isobutyric acid	PT: *M 1.6 T:*M 0.7	PT: *R 1.2–3.0 T:*R 0.3–1.4	μmol/g
	20a (II)		4	Month	Isovaleric acid	PT: *M 1.7 T:*M 0.7	PT: *R 1.5–3.5 T:*R 0.09–1.9	
	20a (III)		4	Month	Valeric acid	PT: *M 0.08 T:*M 0.05	PT: *R 0.0–0.5 T:*R 0.0–0.11	
	20a (IV)		4	Month	Isocaproic acid	PT: *M 0.06 T:*M 0.0	PT: *R 0.0–0.4 T:*R 0.0–0.0	
	20a (V)		4	Month	Caproic acid	PT: *M 0.0 T:*M 0.0	PT: *R 0.0–0.0 T:*R 0.0–0.06	
Wopereis et al. (68)	41a (I)		12	Week	Valeric acid	0.0086	2.6885E-10	μmol/g
	41a (II)		12	Week	Isobutyric acid	0.061600422	0.265002108	
	41a (III)		12	Week	Isovaleric acid	0.21198658	0.487114382	
Brink et al. (36)	4b		9	Month	Valeric acid	13.3 (*M. positive mode)	2.51 (IQR)	log transformed peak areas
	4f		9	Month		13.1 (*M. positive mode)	1.81 (IQR)	
	4j		9	Month		14.6 (*M. positive mode)	3.25 (IQR)	

(Continued)

TABLE 3 | Continued

References	Study ID	Time range	Time when evaluated	Time unit when evaluate day (day/week/month)	Other SCFA name	Concentration (Mean)	Concentration (SD)	Concentration unit
Wopereis et al. (68)	41b (I)	6-9 MONTHS	26	Week	Valeric acid	0.054087559	0.24914562	μmol/g
	41b (II)		26	Week	Isobutyric acid	0.348646926	0.753113276	
	41b (III)		26	Week	Isovaleric acid	0.525732305	0.885977824	
Brink et al. (36)	4c		12	Month	Valeric acid	13.9 (*M. positive mode)	2.54 (IQR)	log transformed peak areas
	4g		12	Month		16.4 (*M. positive mode)	3.65(IQR)	
	4k		12	Month		16 (*M. positive mode)	3.3 (IQR)	
Differding et al. (38)	4l	12	Month		14.9 (*M. positive mode)	2.64 (IQR)		
	8a	9-12 MONTHS	12	Month	Other SCFA Name (*Low abundance SCFAs including isobutyric acid, valeric acid, isovaleric acid, heptanoic acid, and hexanoic acid have been grouped into the "other" category for visual clarity)	3.4	nd	%
	8c		12	Month		4.9	nd	
Nilsen et al. (42)	25c		12	Month	All other (isobutyrate, isovalerate, valerate)	2.5	nd	%
Díaz et al. (37)	7	> 12 MONTHS	12-24	Month	Branched chain fatty acids (BCFAs), isobutyric and isovaleric acids	*MA: 2.59	IQR: 1.94-3.37	μmol/g
Kosuwon et al. (57)	18 (I)		18 (I)	18 (I)	Valeric acid	*M 2.18	*R 0.08-3.12	μmol/g
	18 (II)		18 (II)	18 (II)	Isobutyric acid	*M 2.46	*R 1.75-3.55	
	18 (III)				Isovaleric acid	*M 3.26	*R 2.17-5.28	
Lund-Blix et al. (41)	20b (I)		12	Month	Isobutyric acid	PT: *M 1.8 T:*M 1.55	PT: *R 0.9-2.3 T:*R 0.82-2.42	μmol/g
	20b (II)		12	Month	Isovaleric acid	PT: *M 2.3 T:*M 1.96	PT: *R 0.6-3.2 T:*R 0.92-2.99	
	20b (III)	12	Month	Valeric acid	PT: *M 0.18 T:*M 0.25	PT: *R 0.03-0.27 T:*R 0.07-0.82		
	20b (IV)	12	Month	Isocaproic acid	PT: *M 0.0 T:*M 0.0	PT: *R 0.0-0.0 T:*R 0.0-0.0		
	20b (V)	12	Month	Caproic acid	PT: *M 0.0 T:*M 0.0	PT: *R 0.0-0.0 T:*R 0.0-0.0		

PT, pre-term, T-term; *R, range; *IQR, interquartile range; *M, median.

influence the later production of SCFAs. After birth, the child's microbiota matures and resembles the adult type by 2-3 years of age (80). Characteristics at each stage of development are consistent with the functional maturation of the microbiome. The type of diet has a major influence on the composition and functioning of the microbiota, but it is the cessation of breastfeeding rather than the introduction of solid foods that determines its maturation (1). Our meta-analysis provides evidence that propionic and butyric acid levels change over time

only in breastfed infants. The source of gut microbes in infants is the mother's skin, vagina, stool and breastfeeding by the mother (8, 81-83).

The gut microbiota of infants has been shown to differ between breastfed and formula-fed infants (84-89) and changes rapidly during the transition from breastfeeding to formula-fed feeding (90). Microbiota changes in the first 6 months of life were observed by Ho et al. who conducted a meta-analysis of seven studies examining microbiota changes (1,825 stool samples,

TABLE 4 | Effect sizes (Means) for SCFAs ($\mu\text{mol/g}$) in particular age ranges by feeding type.

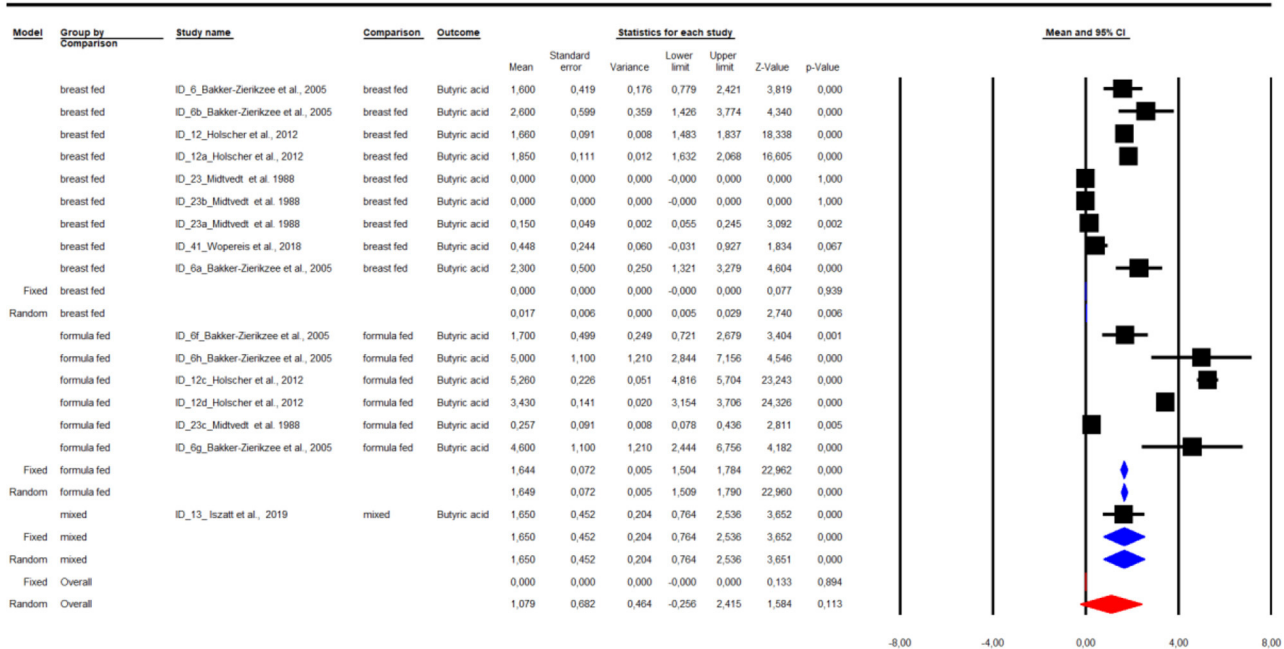
	Time point	Number of studies	Effect size (mean)	SE	Variance	Lower limit	Upper limit	Test Z (z value)	Test Z (p -value)	Q value	df (Q)	p -value	heterogeneity (I^2 * from fixed effect analysis)	
Up to 1 month	Acetic acid													
		Breast fed	8	50.05	5.84	34.12	38.60	61.50	8.57	0.00	4,345.04	7.00	0.00	99.84
		Formula fed	6	46.57	6.78	45.92	33.29	59.85	6.87	0.00	1,305.58	5.00	0.00	99.62
		Mixed	8	22.39	5.86	34.33	10.91	33.87	3.82	0.00	217.01	7.00	0.00	96.77
		Total between									12.86	2.00	0.00	
		Overall	22	39.57	9.34	87.26	21.26	57.88	4.24	0.00	5,896.88	21.00	0.00	99.64
		Propionic acid												
		Breast fed	8	3.69	1.06	1.13	1.61	5.77	3.48	0.00	419.90	7.00	0.00	98.33
		Formula fed	7	11.83	1.21	1.47	9.45	14.21	9.75	0.00	1,639.55	6.00	0.00	99.63
		Mixed	2	3.39	2.08	4.34	-0.69	7.48	1.63	0.10	3.18	1.00	0.07	68.59
		Total between									28.41	2.00	0.00	
		Overall	17	6.39	3.04	9.27	0.42	12.36	2.10	0.04	2,146.24	16.00	0.00	99.25
		Butyric acid												
		Breast fed	9	0.02	0.01	0.00	0.00	0.03	2.74	0.01	679.57	8.00	0.00	98.82
		Formula fed	6	1.65	0.07	0.01	1.51	1.79	22.96	0.00	662.40	5.00	0.00	99.25
	Mixed	1	1.65	0.45	0.20	0.76	2.54	3.65	0.00	0.00	0.00	1.00	0.00	
	Total between									525.49	2.00	0.00		
	Overall	16	1.08	0.68	0.46	-0.26	2.42	1.58	0.11	1,882.53	15.00	0.00	99.20	
	All SCFAs (not specified)													
	Breast fed	5	44.55	4.30	18.51	36.11	52.98	10.35	0.00	63.13	4.00	0.00	93.66	
	Formula fed	5	55.99	4.94	24.42	46.31	65.68	11.33	0.00	42.54	4.00	0.00	90.60	
	Total between									3.05	1.00	0.08		
	Overall	10	50.01	5.72	32.68	38.81	61.22	8.75	0.00	232.55	9.00	0.00	96.13	
1-3 months	Acetic acid													
		Breast fed	5	52.27	12.59	158.43	27.60	76.94	4.15	0.00	2,740.10	4.00	0.00	99.85
		Formula fed	5	54.45	12.59	158.58	29.77	79.13	4.32	0.00	382.12	4.00	0.00	98.95
		Mixed	1	22.60	28.15	792.46	-32.57	77.77	0.80	0.42	0.00	0.00	1.00	0.00
		Total between									1.10	2.00	0.58	
		Overall	11	50.40	8.79	77.29	33.17	67.64	5.73	0.00	3,144.24	10.00	0.00	99.68
		Propionic acid												
		Breast fed	5	7.67	1.81	3.28	4.12	11.22	4.24	0.00	140.69	4.00	0.00	97.16
		Formula fed	5	13.87	1.82	3.31	10.30	17.43	7.63	0.00	31.62	4.00	0.00	87.35
		Mixed	1	3.20	3.69	13.59	-4.03	10.43	0.87	0.39	0.00	0.00	1.00	0.00
		Total between									9.58	2.00	0.01	
		Overall	11	8.79	3.02	9.10	2.88	14.71	2.92	0.00	406.16	10.00	0.00	97.54
		Butyric acid												
		Breast fed	5	3.28	0.76	0.57	1.79	4.76	4.33	0.00	84.44	4.00	0.00	95.26
		Formula fed	5	5.08	0.78	0.61	3.55	6.60	6.51	0.00	10.11	4.00	0.04	60.43
	Total between									2.73	1.00	0.10		
	Overall	10	4.17	0.90	0.81	2.41	5.93	4.64	0.00	362.79	9.00	0.00	97.52	
	All SCFAs (not specified)													
	Breast fed	3	74.58	17.44	304.05	40.40	108.75	4.28	0.00	113.98	2.00	0.00	98.25	
	Formula fed	4	66.48	15.56	241.97	35.99	96.97	4.27	0.00	11.52	3.00	0.01	73.96	
	Total between									0.12	1.00	0.73		
	Overall	7	70.07	11.61	134.74	47.32	92.82	6.04	0.00	151.19	6.00	0.00	96.03	
	Acetic acid													
	Breast fed	5	67.19	9.56	91.42	48.45	85.93	7.03	0.00	1,968.54	4.00	0.00	99.80	
	Formula fed	2	62.95	15.45	238.61	32.67	93.22	4.08	0.00	3.60	1.00	0.06	72.21	
	Total between									0.05	1.00	0.82		
	Overall	7	66.01	8.13	66.09	50.08	81.95	8.12	0.00	2,007.52	6.00	0.00	99.70	
	Propionic acid													
	Breast fed	5	6.70	3.50	12.27	-0.17	13.56	1.91	0.06	939.19	4.00	0.00	99.57	
	Formula fed	2	15.88	5.70	32.48	4.71	27.05	2.79	0.01	4.71	1.00	0.03	78.76	

(Continued)

TABLE 4 | Continued

	Time point	Number of studies	Effect size (mean)	SE	Variance	Lower limit	Upper limit	Test Z (z value)	Test Z (p-value)	Q value	df (Q)	p-value	heterogeneity (I ² from fixed effect analysis)
3-6 months	Total between									1.89	1.00	0.17	
	Overall	7	10.19	4.46	19.88	1.45	18.93	2.29	0.02	1,019.54	6.00	0.00	99.41
	Butyric acid												
	Breast fed	5	2.16	0.61	0.37	0.96	3.36	3.54	0.00	280.55	4.00	0.00	98.57
	Formula fed	2	5.49	1.21	1.46	3.12	7.85	4.55	0.00	0.03	1.00	0.87	0.00
	Total between									6.03	1.00	0.01	
	Overall	7	3.66	1.65	2.73	0.42	6.90	2.21	0.03	322.66	6.00	0.00	98.14
	All SCFAs (not specified)												
	Breast fed	5	2.16	0.61	0.37	0.96	3.36	3.54	0.00	280.55	4.00	0.00	98.57
	Formula fed	2	5.49	1.21	1.46	3.12	7.85	4.55	0.00	0.03	1.00	0.87	0.00
Total between									6.03	1.00	0.01		
Overall	7	3.66	1.65	2.73	0.42	6.90	2.21	0.03	322.66	6.00	0.00	98.14	

Butyric acid up to 1 month of age by feeding type

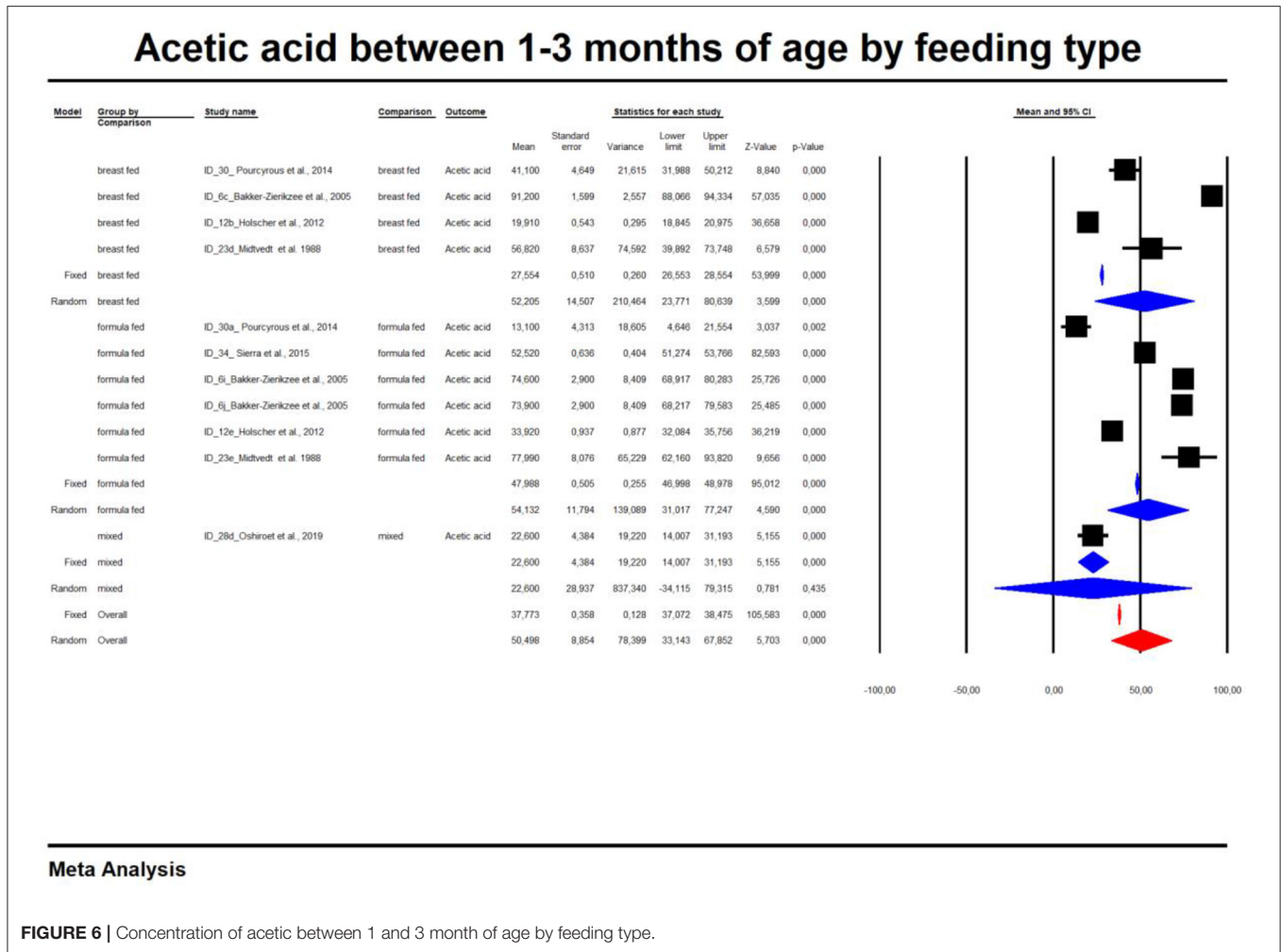


Meta Analysis

FIGURE 5 | Concentration of butyric up to 1 month of age by feeding type.

684 infants) under the influence of feeding (7). In the first 6 months of life, the diversity of intestinal bacteria, the age of the microbiota indicating its maturity, the relative abundance of Bacteroidetes and Firmicutes, and the predicted pathways of carbohydrate metabolism are greater in artificially fed

infants. An increase in the relative abundance of Bacteroidetes and Firmicutes and of bacteria at lower taxonomic levels was observed in artificially fed infants (order: *Bacteriales*, *Clostridiales*, families: *Bacteroidaceae*, *Veillonellaceae*; genera: *Bacteroides*, *Eubacterium*, *Veillonella* and *Megasphaera*)



Meta Analysis

FIGURE 6 | Concentration of acetic between 1 and 3 month of age by feeding type.

compared to breastfed infants (91) and/or a tendency toward increased bacterial diversity in infants, regardless of the type of diet (92–94). These observations could explain why infants fed with artificial infant formula have higher levels of SCFAs in their stools. However, it should be emphasized that the studies we included in our work were characterized by considerable heterogeneity, which also explains the high heterogeneity of the results of our meta-analysis. It is reasonable to assume that a more stable, less differentiated gut microbiota is present in the context of breastfeeding during the first months of development (7) and that changes in the microbiota caused by artificial feeding may affect children’s health later in life. While *Bifidobacterium* is the most common bacterial species in the digestive tract of infants, *Bacteroides* and *Eubacterium* are the most common bacterial species in the intestines of adults (95, 96). While these genera may be part of the normal environment of gut bacteria, an increase in *Bacteroides* in the gut has been shown to be associated with higher body mass index (BMI) in young children (97), while *Veillonella* may be associated, at least to some extent, with various types of infections (98). The increase in the relative abundance of major metabolic pathways related to

carbohydrate metabolism in infants fed artificial infant formula and the decrease in the relative abundance of major metabolic pathways related to lipid metabolism/homeostasis, free radical detoxification, and cofactor and vitamin metabolism, may also be associated with a higher risk of obesity, diabetes and other adverse health outcomes in children who were not breastfed early (99–101). From our perspective, the results of the Ho et al. meta-analysis are particularly important because it included children from different geographic regions, which may have had an impact on the microbiota (7).

Observations on the relationship between microbiota composition and stool SCFAs concentration are scarce. Tsukuda et al. (102) observed in 12 children during the first 2 years of life three distinct phases of progression of SCFA profiles: the early phase with a low acetate content and high succinate content, the intermediate phase with a high lactate and formate content, and the late phase with a high propionate and butyrate content. The assessment of the relationship between SCFA and the microbiota showed that the presence of butyrate in the feces is associated with an increased number of *Clostridiales* and the cessation of breastfeeding and that a diverse and

individualized set of *Clostridiales* species harboring acetyl-CoA pathway plays a significant role in the production of butyrates in the intestine. The early microbiome showed significant variation in composition and progression and varied over time. *Enterobacteriales* typically dominated the newborn microbiota, but their numbers declined with age. *Bifidobacteriales* constituted the main bacterial order in early-life microbiota, showing an increasing abundance up to 6 months of age and a decrease after 8 months of age. *Clostridiales* were less numerous until 8 months of age and then increased. It was also confirmed that α -diversity increased with age. At the end of the study period, such elevation rates slowed down, although, in 2-year-old infants, this index was still significantly lower than for their parents. The day of transition showed significant inter-individual variability; the transition from *Enterobacteriales* to *Bifidobacteriales* lasted from 3 days to 6 months (median, 0.6 months), while the transition time from *Bifidobacteriales* to *Clostridiales* ranged between 8 and 24 months (median, 13 months). Consistent with previous studies (2, 103), the transition from a *Bifidobacteriales*-dominated microbiota to *Clostridiales*-dominated one has been associated with cessation of breastfeeding. These observations explain the previously mentioned heterogeneity of the observed results. As with the gut microbiota development, intestinal SCFAs synthesis profiles during early life were dynamic and individualized, with patterns showing temporal trajectories.

The major SCFA throughout the study period was acetate; its concentration increased to 6 months and then remained at a constant level. The concentration of propionate and butyrate increased after 8 and 10 months of age, respectively. Branched-chain fatty acids (i.e., isobutyrate and isovalerate) were rarely detected throughout the study (especially up to 9 months of age). The authors divided the SCFA pattern into three types, characterized by either low acetates and high succinate (SCFA type 1), high lactate and high formate (SCFA type 2), or high propionate and high butyrate (SCFA type 3). They then summarized the progression of the SCFA type production and observed sequential shifts from SCFA types 1 to 2 and then to type 3, with individual variability on the day of transition. The transition from the type 2 to the type 3 SCFA profile was dependent on the increase in propionate concentration observed prior to cessation of breastfeeding, while the increase in butyrate and decrease in lactate and formate coincided with the cessation of breastfeeding. A relationship was also found between SCFAs and microbiota profiles, but these relationships were inconclusive. The effect of human milk on gut bacteria may be related to large amounts of lactoferrin (104), which limits the availability of iron ions necessary for bacteria to carry out enzymatic reactions and regulate gene expression (105). *Bifidobacteria* species are well adapted to conditions with low iron content (106), and lowering its availability results in decreased butyrate production (107, 108). Discontinuation of breastfeeding may result in a decrease in the amount of lactoferrin and an increase in the amount of free Fe, thus leading to increased production of butyrate in the intestines.

A comparison of SCFA fecal concentration between breastfeeding vs. formula feeding in age was presented in **Table 5**. Brink et al. observed that the levels of butyric acid in

the feces of breastfed infants were higher than in formula-fed infants (36), which can prevent infections during this period of life (109). In children aged 3–5 months, it has been found (110) that exclusive breastfeeding was associated with lower absolute concentrations of total SCFAs, acetate, butyrate, propionate, valerate, isobutyrate, and isovalerate but higher concentrations of lactate. Moreover, the relative proportion of acetate was higher in the case of exclusive breastfeeding. This association was independent of the mode of delivery, intrapartum antibiotics administration, infant sex, age, site of enrolment, and maternal BMI or socioeconomic status.

Edwards et al. also found an elevation in the relative proportions of acetate (76%) in breastfed infants at 4 weeks of age (32). The higher relative acetate abundance in exclusively breastfed infants may be partly due to the presence of HMO in breast milk that is not present in the infant formula. HMOs, the third largest component of human milk, are soluble carbohydrates that are not digested by the host and are substrates for selected gut microbiota (111). There is a close relationship between the infant's gut microbiota, that of the mother's milk, and the composition of human milk oligosaccharides (HMO) (112–115). Recent studies have shown that human milk microbiota can directly colonize an infant's intestines, and the effect of human milk on the infant's intestinal microbiota is dose-dependent (8). The microbiota in breast milk changes during lactation and is different in exclusively breastfeeding and non-breastfeeding mothers (116, 117). The abundance of gut microbiota in breastfed infants, especially *bifidobacteria* species, is correlated with maternal HMO content and its catabolic activity (82, 118, 119). *Bifidobacterium* species are a narrow group of gut bacteria capable of metabolizing HMO (120) and are therefore over-represented in the microbiota of breastfed infants compared to formula-fed babies (1, 93, 121). The results of studies by Azad et al. showed that the *Bifidobacteriaceae* family is enriched in the case of breastfeeding (93).

Bifidobacterium has been shown to metabolize HMO to produce acetate and lactate (122, 123). Martin et al. observed that the increase in the number of *bifidobacteria* corresponds to the increase in the acetate concentration in the stool (124). Although evidence is limited, higher acetate levels in breastfed infants may protect against gut pathogens and allergic diseases (125). Fukudo et al. showed that acetate, produced by *bifidobacteria*, improved intestinal defenses and protected against *Escherichia coli* O157: H7 in mice (122). Thorburn et al. (126) proposed that the inhibition of HDAC by acetate, shown in an adult mouse model, increases the transcription of the *Foxp3* gene, which may promote Treg- suppression of airway inflammation and induction of oral tolerance. In infants, Arrieta et al. found that a decreased amount of acetate in the stool at 3 months of age was associated with allergic disease in later infancy (127).

Other components of breastmilk can also affect gut microbiota development (128, 129). For instance, maternally derived antibodies: IgA, IgM, IgG and secretory IgM (SIgM) and IgA (SIgA) have the ability to bind the microbes and consequently protect against respiratory and gastrointestinal infections (130). Cytokines and growth factors are mother-to-child signals synthesized to improve action of leukocytes. What is more,

TABLE 5 | Comparison of SCFA fecal concentration between breastfeeding (B) vs. formula feeding (F) including data on analytical methods and formula composition.

Reference	SCFAs analytical method	Microbiota analysis/ method	Formula composition	Comparison of SCFA fecal concentration between B vs. formula feeding F during the study time	Comparison of SCFA fecal concentration between BF vs. formula FM in age
Ben et al. (29)	GC	Culture	No	No	3 month: acetic acid: BF > FM.
Ben et al. (30)	GC	Culture	No	No	3, 6 month: acetic acid: BF > FM.
Berni Canani et al. (48)	GC	16SrRNA, V3-V4.	NA	NA	NA
Brink et al. (36)	UHPLC/MS(Q-Exactive Hybrid Quadrupole-Orbitrap).	16SrRNA, V4.	No	No	3, 6 month: Butyric acid: BF > FM (also 9 month); Propionic acid: BF < FM; Isovaleric acid: BF > FM; Isobutyric acid: BF < FM.
Bazanella et al. (52)	UHPLC/Q-ToF-MS.	16SrRNA,V3-V4/ strain identification - PCR.	Yes	Concentrations of propionic, butyric, isovaleric, and valeric acid increased over time in B and F group.	BF vs. FM: lower proportions of propionate, butyrate, valerate, isovalerate and higher pyruvic and lactic acid.
Bakker-Zierikzee et al. (31)	GC	FISH	Yes	No	5, 10 days; 4, 8, 12, 16 weeks: no difference of Acetate, Propionate, Butyrate, sum of isobutyrate, isovalerate and valerate SCFA between BF v FM.
Díaz et al. (37)	GC	16SrRNA, V3-V4.	NA	NA	NA
Differding et al. (38)	GC	16SrRNA, V4.	NA	NA	NA
Edwards et al. (32)	GLC	No	Yes	No	2, 4 weeks: Butyric acid, Propionic acid, Isovaleric acid: BF < FM; Acetic acid: BF = FM.
Fleming et al. (53)	HPLC	16SrRNA	NA	NA	NA
Heath et al. (33)	GC	NA	NA	NA	NA
Holscher et al. (54)	GC/MS	FISH	Yes	No	0, 3, 6 weeks: acetate, propionate, and total SCFAs: BF < FM; isobutyrate BF > FM.
Iszatt et al. (39)	GC	16SrRNA, V4.	NA	NA	NA
Kien et al. (40)	GLC	NA	NA	NA	NA
Kim et al. (55)	1H-NMR spectroscopy	NA	NA	NA	NA
Knol et al. (56)	GC	FISH	Yes	NA	AN
Kok et al. (34)	GC	16SrRNA	Yes	0→ 30 day: propionate: increase of the concentration for amino acid formula; 0→ 60 day: butyrate and propionate: increase of the concentration for extensively hydrolyzed formula and amino acid formula. No changes in concentration for BF	30, 60 day: butyrate, propionate: FM (extensively hydrolyzed and amino acid formula) > BF; 60 day: total SCFA: F (amino acid formula) > BF.
Kosuwon, et al. (57)	GC	FISH	Yes	NA	NA
Liu et al. (58)	GC	PCR	Yes	No	3 weeks: Acetic acid FM < BF, Propionic acid and Butyric acid FM = BF.
Lund-Blix et al. (41)/ Dahl et al. (27) ^a	No	16SrRNA, V4.	NA	NA	NA
Maldonado et al. (59)	GC	Culture/PCR.	NA	NA	NA
Mentula et al. (60)	GC	Culture	NA	NA	NA
Midtvedt et al. (49)	GLC	NA	NA	NA	NA
Mohan et al. (61)	GC	Culture	NA	NA	NA
Nilsen et al. (42)	ND	16SrRNA, V4/ shotgun/ metaproteomics	NA	NA	NA

(Continued)

TABLE 5 | Continued

Reference	SCFAs analytical method.	Microbiota analysis/ method.	Formula composition.	Comparison of SCFA fecal concentration between B vs. formula feeding F during the study time.	Comparison of SCFA fecal concentration between BF vs. formula FM in age.
Nocerino et al. (62)	GC	16SrRNA	NA	NA	NA
Nogacka et al. (43)	GC	16SrRNA, V3.	NA	NA	NA
Oshiro et al. (63)	ND	reverse-transcription quantitative PCR	NA	NA	NA
Park et al. (44)	GC-MS	16SrRNA, V1-V3/shotgun.	ND	ND	ND
Pourcyrus et al. (50)	IC	No		Preterm infants: 0→ 80 days: Butyrate: B: ↓, F: ↑; Propionate: B: ↑ > F: ↑	Preterm infants: acetate, propionate, total SCFA: BF > FM
Quin et al. (35)	GC	16SrRNA, V3-V4.	NA	NA	NA
Rao et al. (45)	GC-MS	16SrRNA, V3-V4.	NA	NA	NA
Roduit et al. (46)	HPLC	No	NA	NA	NA
Sierra et al. (64)	GC	PCR	Yes	NA	NA
Stansbridge et al. (65)	GC	No	NA	NA	NA
Ta et al. (47)	LC/MS/MS	Metagenomic/metatranscriptomic	NA	NA	NA
Tauchi et al. (51)	HPLC	16SrRNA, V1-V2.	NA	NA	NA
Underwood et al. (66)	HPLC	Culture/PCR	NA	NA	NA
Westerbeek et al. (67)	GC	FISH	NA	NA	NA
Wopereis et al. (69)	GC	16SrRNA, V3-V4	Yes	NA	8 week: acetate, propionate, butyrate, iso-valerate, iso-butyrate : BF = FM.
Wopereis et al. (68)	GC	16SrRNA, V3-V4	Yes	NA	4, 12, 26 weeks: proportions propionate, butyrate, isobutyrate and isovalerate BF < FM.

GC, gas chromatography; UHPLC, ultra-high performance liquid chromatography; MS, mass spectrometry; HPLC, high performance liquid chromatography; Q-ToF, quadrupole time of flight; 1H-NMR, proton nuclear magnetic resonance; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; GLC, gas liquid chromatography; IC, ion chromatography; LC, liquid chromatography; ND, no data; NA, not applicable. ^asource of data indicated by Lund-Blix et al. (41).

defensins and cathelicidins that breast milk contains are well-known antimicrobial molecules (131). Paralelly, breast-milk originated lysozyme breaks the microbial cell walls *via* lysis process. Free amino acids which are present in breast milk are essential for growth of the nervous tissue, eye and intestines and protein breaking enzymes support the hydrolysis of the milk proteins (132). Breast milk also stimulates the secretion of calprotectin and zonulin, both involved in gut barrier and microbiota development (133).

We have also observed lower absolute concentrations of SCFAs in exclusively breastfed infants, which is in line with other published studies. In 111 stool samples analyzed by NMR, Martin et al. found lower concentrations of SCFAs at 3 and 6 months of age in breastfed infants born to overweight or obese mothers (134). In a small study of 4 infants using GC and LC mass spectrometry, valerate and isovalerate concentrations were higher in formula-fed infants, the latter more than 40 times higher than in breastfed infants (135). The higher absolute concentrations of SCFAs observed in formula-fed infants may result from the greater bacterial diversity observed in these infants compared to purely breastfed

infants (93) and thus a greater ability to metabolize substrates present in the intestines. The exclusivity of breastfeeding was inversely related to both the richness and diversity of the microbiota (93). The observed differences may also result from differences in the composition or absorption of breast milk compared to the modified milk and thus from differences in the availability of substrates. In addition, higher concentrations of branched-chain fatty acids, valerate, isobutyrate, and isovalerate, derived from amino acid metabolism, indicate reduced protein absorption or excessive protein intake [potentially due to the higher protein content of the formula compared to human milk (136) in formula-fed babies]. The availability of these substrates is likely to increase the abundance of proteolytic bacteria, such as *Bacteroides* and *Clostridia*, seen in formula-fed infants (93). Higher concentrations of proteolytic metabolites in mineral formula-fed infants may also be due to the reduced availability of carbohydrates without HMO and, thus, greater energy extraction from protein metabolism. Chow et al. (135) showed that in the absence of fermentable carbohydrates in the feces of both breast- and formula-fed infants, mainly metabolites were produced, indicating fermentation of proteins; their production

was reduced with the addition of various fermentable HMO-like carbohydrate substrates. Increased fecal SCFAs in formula-fed infants may have metabolic consequences. Several studies have reported higher fecal SCFAs in adults and overweight children compared to their lean counterparts (137–141) and correlations with other metabolic risk factors (141). While causality has not yet been established, the authors of these studies hypothesize that a higher concentration of SCFAs may reflect the increased ability of the gut microbiota to obtain energy from the diet.

Our meta-analysis also had some limitations. We observed significant heterogeneity in the papers analyzed due to different analytical methods. For the same reason, we did not compare the results obtained to the gut microbiota composition. In addition, the authors of the papers did not often provide the composition of the dietary mixtures. All this information is included in **Table 5**. Another limitation of our study was the indirect measurement of luminal metabolites by analyzing stool samples. The concentration of metabolites in the feces is a function of production, absorption, use by other microorganisms, and stool transit time. Few studies have examined whether the content of SCFA in stool is a reliable indicator of luminal content, especially in early childhood, although it is estimated that 95% of SCFA produced in the gut is rapidly absorbed, and only 5% is excreted in the feces (15). In a study of healthy adults, Vogt and Wolever found that fecal acetate concentrations are inversely correlated with acetate absorption, suggesting that fecal acetate concentrations may reflect intestinal absorption rather than production (142). However, given that the potential of fecal metabolite analysis is to provide a biomarker for predicting future disease risk, analysis of stool samples provides a non-invasive and cost-effective method for epidemiological cohort studies. Our sampling was also only at a one-time point in infancy; therefore,

our data do not provide comprehensive information on trends in metabolites over time concerning diet. A limitation of our work is the inability to assess the long-term health consequences of SCFAs stool concentration.

We can conclude that the feeding mode influences the content of SCFAs in stools in early life, with artificial feeding being associated with a higher content of SCFAs in stools and breastfeeding with the occurrence of more significant differences in the concentration of SCFAs in stools between different periods of a child's early life. There is a need for long-term assessment of the impact of observed differences on health in later life and unification of analytical methods and methodologies for SCFAs testing in young children.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

IŁ and BŁ: conceptualization. KS-Ż: methods and statistical analysis. LS, MF-T, and PT: investigation. IŁ: original draft. All authors: review and editing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.939194/full#supplementary-material>

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