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Faecal Short-chain Fatty Acid and Early Introduction of Foods in the First 200 Days of Infant's Life in the District of Abidjan (Ivory Coast)

Mouroufie Abo Kouadio Jerome[1,2,*], Silué Fatogoma Etienne[3], Coulibaly Ngolo David[1,2], Muriel Thomas[4], PEPKE Fréderic[4], Coxam Véronique[5], Kati-Coulibaly Seraphin[1], FAYE-KETTE Hortense[1,2]

[1]Laboratory of Biology and Health, Faculty of Biosciences, University Felix Houphouet-Boigny, Abidjan, Ivory Coast 22 B.P. 582 Abidjan 22, Ivory Coast
[2]Institut Pasteur de Cote d'Ivoire, Abidjan, Ivory Coast
[3]Training and Research Unit, Agriculture Fisheries Resources and Agro-industry, University of San pédro, Cote d'Ivoire
[4]Université Paris-Saclay, INRAE, Micalis, Jouy-en-Josas, France
[5]University Clermont Auvergne, INRAE, UNH, Human Nutrition Unit, Clermont-Ferrand, France
*Corresponding author: abojerome7@gmail.com

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Abstract Dosage of short chain fatty acids (SCFAs) according to food diet showed that the content of acetate was high in newborn feaces. Infants receiving food supplements have a complex and diverse gut microbiota. Moreover, the results show that infants from poor districts have an abundant concentration of SCFAs in their faeces compared to those living in places with a relatively high standard of living. Among infants receiving milk, the highest proportion of SCFA is acetate in breastfed infants (BF) at a rate of 15.025 ± 2.23 µmol/g, followed by propionate in infants receiving mixed feeding (BF+FF), at a rate of 13.58 ± 1.03 µmol/g and butyrate in infants taking mixed feeding at a rate of 0.32 ± 0.72 µmol/g. However, among infants starting early diet diversification, acetate is higher in infants receiving milk formula and diet diversification (FF+FD) with a concentration of 25.4 ± 0 µmol/g, followed by propionate (2.36 ± 0 µmol/g) in infants receiving mixed feeding (BF+FF) and butyrate in those fed with (BF+FD). Partial breastfeeding is associated with a higher proportion of acetate, butyrate and propionate. The study of the correlation between the different SCFAs produced and the ASV (Variants of Microbial Amplicon Sequences) of the intestinal community of the child, shows that acetate is positively correlated with *Bifidobacterium* and negatively with *Streptococcus* and *Escherichia-Shigella*. Propionate is positively correlated with *Bifido bacterium* and negatively with Escherichia-Shigella. Similarly, butyrate is positively correlated with *Bifidobacterium* and negatively with *Escherichia-Shigella*.

Keywords: *infants, early diet diversification, gut microbiota*

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1. Introduction

The gut microbiota provides a number of important functions, like the metabolism of food fibers and other non digested macronutrients by the host. The main products of this fermentation process are short chain fatty acids (SCFAs) and other intermediate metabolites such as lactate and succinate. The production of these metabolites depends on both the diet and the content of the intestinal microbiota. There is growing evidence for the role of SCFAs in host physiology and metabolic processes as well as in chronic inflammatory conditions such as allergic diseases and obesity. These fibers help to increase the biomass [1] of some beneficial bacteria (bifidobacteria, lactobacillus, etc.) [2]. This intestinal flora has an impact on account of its activity on the gastrointestinal transit of the bolus.

The early introduction of some foods during infancy will not only influence the content of the faecal microbiota and the concentration of SCFAs produced. Similarly, the type of diet can modulate the production of SCFAs and/or the abundance of SCFA-producing bacteria. For instance, food diets rich in fibers or food diets rich in omega-3s has been correlated with higher levels of SCFAs and SCFA-producing bacteria [3,4].

Today, the majority of studies are focused on the relationship between protein intake and SCFA production and were carried out on old people. Furthermore, other studies were undertaken on SCFAs, food diets and their links with the content of the gut microbiota, [5,6]. However, very few observational studies are currently available comparing the production of SCFAs in different population groups with different food diets for infants, despite the early introduction of foods.

In addition, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) exclusive recommend breastfeeding for up to six months. In spite of these recommendations, it is common to see early addition of food supplements in infants' diet. Current feeding practices do not do not match with steps needed to support exclusive breastfeeding. Indeed, at three months of age, the majority of infants are fed with food supplements [7]. However, very few studies provide relevant information on faecal SCFA concentration in infants starting early diet diversification. These common practices can lead to the intestinal microbiota imbalance and the release of SCFA by-products such as ammonia, phenol, p-cresol or biogenic amines that can damage the intestinal cells [8,9].

The objective of this study is to assess faecal SCFA concentration in infants according to age, sex, place of residence and type of diet.

2. Materials and Methods

2.1. Study Population

The study sample included 50 subjects as followed: 21 males and 26 females, with an age ranging from zero to 6 months (Table 1). All the children were recruited in the District of Abidjan (Abobo, Cocody and Marcory) and their mothers were between 18 and 40 years old. For descriptive purposes, the age of children was divided into three age groups according to the age of early food introduction (0–119 days), (120-179 days), and 180-200days. Ethical approval was obtained from the National Health and Life Sciences Ethic Committee. All volunteers, or their legal representatives, gave a written informed consent. The exclusion criteria for this study were not to be diagnosed with diseases and not having consumed antibiotics or probiotics during month of recruitment. All measurements were carried out in accordance with approved guidelines and regulations.

Table 1. Characteristics of study samples

Sample name	District	Age	Gender	Food	Sample name	District	Age	Gender	Food
Infant 1	Marcory	75 days	M	BF	**Infant 26**	Marcory	195 days	F	BF + FF
Infant 2	Cocody	60 days	F	BF +FF	**Infant 27**	Abobo	195 days	M	BF + FD
Infant 3	Cocody	90 days	F	FF	**Infant 28**	Abobo	190 days	F	BF + FF
Infant 4	Marcory	120 days	M	BF	**Infant 29**	Abobo	45 days	F	BF +FD
Infant 5	Marcory	60 days	F	BF	**Infant 30**	Abobo	195 days	F	BF + FD
Infant 6	Cocody	60 days	F	BF	**Infant 31**	Marcory	185 days	M	BF + FF
Infant 7	Cocody	60 days	F	BF +FF	**Infant 32**	Abobo	90 days	F	BF
Infant 8	Marcory	120 days	M	BF	**Infant 33**	Cocody	30 days	F	BF + FF
Infant 9	Cocody	150 days	F	BF +FF	**Infant 34**	Marcory	195 days	F	FF
Infant 10	Cocody	90 days	M	BF +FD	**Infant 35**	Abobo	21 days	F	BF +FD
Infant 11	Cocody	180 days	M	BF +FF	**Infant 36**	Marcory	195 days	F	BF +FD
Infant 12	Marcory	90 days	M	BF	**Infant 37**	Abobo	150 days	M	BF +FF+FD
Infant 13	Cocody	120 days	F	BF + FD	**Infant 38**	Abobo	120 days	M	BF +FF+FD
Infant 14	Marcory	150 days	F	BF + FF	**Infant 39**	Cocody	95 days	M	BF +FF+FD
Infant 15	Cocody	75 days	M	BF +FF	**Infant 40**	Abobo	120 days	M	BF +FF+FD
Infant 16	Cocody	60 days	F	BF + FF	**Infant 41**	Marcory	180 days	F	BF +FF+FD
Infant 17	Marcory	165 days	F	BF +FF+FD	**Infant 42**	Abobo	180 days	F	BF + FD
Infant 18	Abobo	90 days	M	BF + FF	**Infant 43**	Marcory	90 days	F	BF +FF+FD
Infant 19	Abobo	187 days	M	BF + FD	**Infant 44**	Abobo	90 days	M	BF +FF+FD
Infant 20	Marcory	90 days	M	BF	**Infant 45**	Marcory	120 days	F	BF +FF+ FD
Infant 21	Marcory	180 days	F	BF + FD	**Infant 46**	Abobo	45 days	F	FF
Infant 22	Abobo	180 days	F	BF	**Infant 47**	Abobo	135 days	F	BF +FD
Infant 23	Abobo	195 days	M	BF + FF+FD	**Infant 48**	Marcory	150 days	M	BF +FD
Infant 24	Abobo	195 days	M	BF + FF+FD	**Infant 49**	Marcory	120 days	M	FF+FD
Infant 25	Abobo	190 jours	F	BF + FF+FD	**Infant 50**	Abobo	150 days	F	BF +FD

M: Male; **F:** Female
BF= Breast milk
BF+FD = Breast milk + Food Diversification;
FF= Milk formula
FF+FD= Milk formula +Food Diversification
BF+FF=Breast milk + Milk formula
BF+FF+FD= Breast milk + milk formula + Food Diversification

2.2. Faecal Collection and Analysis of Metabolites

Faecal sample collection was performed as described in previous studies [10,11,12]. Briefly, faeces were collected in sterile containers then brought to liquid nitrogen for 5 to 10 minutes and immediately sent to the laboratory and frozen at -20°C. Then, 1 g of faecal samples was diluted (1/10) in Milli-Q water. The supernatant was obtained by centrifugation (10,000 rpm 10 min, 4°C), filtered using filters, adding 2-ethyl butyric acid 1/10 (1 mg/mL) as internal standard and stored at -80°C for analysis. SCFAs were identified and quantified in a gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, France).

2.3. Sample Preparation

One (1) g of stool was collected and wetted with Milli-Q water, homogenized, stirred and kept for 2 hours at 4°C. Then, centrifuged at 12000g for 15 minutes at 4°C. Finally, the supernatant was collected and weighed.

In a chemical fume hood, 40 µL of phosphotungstic acid (Sigma, ref. P4006 - 50g diluted in 20 mL of MilliQ water and stored at 4°C) were added and left to stand overnight at +4°C.

The sample was then centrifuged at 12000g for 15 minutes at 4°C.

Then, the entire supernatant was recovered, weighed and frozen at -20°C for further use.

On the day of the assay, the sample is thawed and centrifuged at 12000 g for 15 minutes at 4°C.

Then, 40 µL of the supernatant was placed in a 2 mL vial (Agilent, ref. 11090500) with a volume reducer (Agilent, ref: 6090357) for GC-FID.

Then, 10 µL of 2-ethyl butyrate was added as an internal standard (Sigma, 2-EthylButyric Acid, 99%, ref: 109959 - 127 µL) diluted in 50 mL of Milli-Q water in a volumetric flask to a final concentration of 20 mM and stored at 4°C.

Finally, a Standard containing AGCC (Sigma, Volatile Free Acid Mix, ref. CRM46975- 40 µL diluted V/V with Milli-Q water for a final concentration of 5 mM in which 10 µL of internal standard) was added [13].

2.4. Statistical Analysis

Statistical analysis of all results was performed using OpenLab Chemsation Agilent software. SCAF means were used to perform principal component analysis (PCA) of SCAF production with STATISTICA, while correlation analysis was studied with R-Studio software version 1.1.463. To investigate correlations between SCFAs and ASVs of gut microbiota, we fitted univariate and multivariate generalized linear regression models to examine the association of the introduction of food supplements with infant faecal SCFA concentrations. and the composition of the intestinal microbiota. After finding out whether ASVs were significantly associated with food diet, we calculated Spearman's correlations on relative abundance of significant ASVs with major SCFAs and total SCFA concentrations in each sample [14].

2.5. Ethical Considerations

Authorization (N/Ref:128-18MSHP/CNESVS-km) was granted by the National Ethics Committee for Life Sciences and Health (CNESVS) of Côte d'Ivoire before this study was carried out.

3. Results

3.1. Distribution of SCFAs

Complementary foods as well as milk diets are sources of dietary fiber, starch and oligosaccharides, as well as carbohydrates that escape digestion in the small intestine. They are fermented in the intestine producing short chain fatty acids (SCFAs). To associate the presence of the bacterial community producing SCFAs with the increase in the concentration of SCFAs in the faecal samples, the levels of acetate, butyrate and propionate were determined by Gas Chromatography. The study of essential short-chain fatty acids shows that in the faeces of infant's acetate is higher regardless of the diet. However, the proportions vary from diet to diet. Thus, the proportions are BF (15.025 ± 2.23 µmol/g); FF (13.18 ± 3.48 µmol/g); BF+FF (13.58 ± 1.03 µmol/g); BF+FD (12.31 ± 0.07 µmol/g); FF+FD (25.4 ± 0 µmol/g); BF+FF+FD (16.57± 8.59 µmol/g) (Table 2).

Table 2. Distribution of fecal short-chain fatty acids

AGCC (µmol/g)	Acetate	Propionate	Butyrate
BF	15,025 ± 2,23	2,79 ± 2,11	0,17 ± 0,47
FF	13,18 ± 3,48	1,48 ± 0,85	0,16 ± 0,19
BF+FF	13,58 ± 1,03	2,45 ± 2,29	0,32 ± 0,72
BF+FD	12,31 ± 0,07	0,52 ± 0,33	0,02 ± 0,03
FF+FD	25,4 ± 0	2,36 ± 0	0±0
BF+FF+FD	16,57 ± 8,59	0,89 ± 0,65	0±0,08

3.2. Determination of Short-chain Faecal Fatty Acids According to Sex

The results show that there is no significant difference between SCFAs and gender ($p > 0.05$). The production of propionate is correlated with the sex of infants. The results of the present study show that SCFAs are abundant in male faeces than in females. However, acetate is more produced (32.18 ± 14.93µmol/g) in males than in females (32.18 ± 14.93 µmol/g). Similarly, propionate is higher (4.74 ± 0.15 µmol/g) in males than in females (3.147 ± 1.60 µmol/g). Butyrate is weakly produced in both males (0.13 ± 0.3) and females (0.28 ± 0.65 µmol/g) (Table 3).

3.3. Faecal Short-chain fatty Acids According to Place of Residence

There is no significant difference between the different SCFAs and the place of residence ($p>0.05$). SCFAs are higher in infants' faeces living in Abobo, followed by those of Marcory and then Cocody. However, the production of acetate and propionate are higher in the district of Abobo than in the other districts. The acetate in Abobo is (34.84 ± 12.26), against 21.20 ± 3.95µmol/g in Marcory

and 11.49 ± 1.06 µmol/g in Cocody. The propionate is 8.55 ± 3.96 µmol/g in Abobo, 3.22 ± 1.67 µmol/g in Marcory and 4.06 ± 0.67 µmol/g in Cocody (Table 4).

3.4. Faecal short-chain Fatty Acids According to Food Typology

Basically, acetate is abundant in all diets in this study, followed by propionate and butyrate.

The results show that there is no significant difference between acetate, propionate in children taking the various diets; FF; BF+FF; BF+FF+FD ($p>0.05$). SCFAs are relatively abundant in infants' faeces starting early food diversification, compared to those who did not receive any food supplements. However, acetate appears to be more abundant in infants' faeces taking FF+FD (25.4±0), followed by BF+FF+FD (16.57 ± 8.59 µmol/g) and BF (15.025 ± 2.23 µmol/g).

There is also no significant difference between the propionate produced in the different diets such as; FF; BF+FF; BF+FF+FD ($p>0.05$). The results show that propionate is more abundant in infants' faeces fed with BF (2.79 ± 2.11µmol/g), followed by BF+FF (2.45 ± 2.29µmol/g) and FF+FD (2.36 ± 0µmol/g). But low in infants consuming BF+FD (0.52 ± 0.33µmol/g).

As for the butyrate, a significant difference was noticed between the production of butyrate and the following diets; BF (0.17 ± 0.47); FF (0.16 ± 0.19µmol/g) vs BF+FF (0.32 ± 0.72µmol/g); BF+FD (0.02 ± 0.03µmol/g) vs FF+FD (0±0µmol/g); BF+FF+FD (0± 0.08µmol/g) (Table 5).

3.5. Faecal Short-chain Fatty Acids According to Age

The results of the analysis of SCFAs production in infants' faeces showed that there is a significant difference in butyrate concentration production ($p < 0.05$) and the different age groups. SCFAs production according to infant age showed that SCFAs are more abundant in infant faeces with ages ranging from 120 to 179 days, followed by those between 0 to 119 days and finally those of 180-200 days of age. Likewise, butyrate concentration appears to be more abundant in infant faeces aged between 4 to 5 months (0.21±0.12µmol/g) than in infants aged 0-3 months (0.11± 0.49 µmol/g) and 6 months (0.04 ± 0.07µmol/g). However, there is no significant difference between the production of acetate and propionate and the different age groups. However, acetate and propionate are abundant in infant faeces aged 0-119 days (Table 6).

Table 3. Faecal short-chain fatty acids according to gender

SCFAs (µmol/g)	Acetate	Propionate	Butyrate	isobutyrate	isovalerate	valerate	isocaproate	caproate
Gender	p = 0.78	p = 0.02	p = 0.6359	p = 0.48	p = 0.8389	p =0.73	p =0.4227	p = 0.247
Females	18.85 ± 6.31[a]	3.147±1.60[a]	0.28±0.65[a]	0.80±0.28[a]	0.57±0.58[a]	0.07±0.07a	0.18±0.18[a]	0±0[a]
Males	32.18 ± 14.93[a]	4.74±0.15[a]	0.13±0.3[a]	0.84±0.08[a]	0.12±0.12 [a]	0.03±0.03[a]	0±0 [a]	0±0 [a]

The results are presented as mean ± deviation. Different letters (a, b, c) indicate significant differences between age groups (P<0.05).

Table 4. Faecal short-chain fatty acids according to place of residence

SCFAs (µmol/g)	Acetate	Propionate	Butyrate	Isobutyrate	Isovalerate	Valerate	Isocaproate	Caproate
Districts	p=0.517	p=0.531	p= 0.856	p=0.781	p= 0.963	p= 0.924	p= 0.783	p=0.714
Abobo	34.84±12.26[a]	8.55±3.96 [a]	0.14±4.79[a]	5.33±0.09[a]	0.13±0.13[a]	0.28±0.28[a]	0±0[a]	0±0[a]
Marcory	21.20±3.95[a]	3.22±1.67[a]	0.11±0.49[a]	0.65±0.11[a]	0.12±0.12[a]	0.03±0.03[a]	0±0 [a]	0±0 [a]
Cocody	11.49±1.06[a]	4.06±0.67[a]	0.28±0.65[a]	0.8±0.28[a]	0.57±0.57 [a]	0.07±0.07[a]	0.18±0.18[a]	0±0 [a]

The results are presented as mean ± deviation. Different letters (a, b, c) indicate significant differences between age groups (P<0.05).

Table 5. Faecal short-chain fatty acids according to food type

SCFAs (µmol/g)	Acetate	Propionate	Butyrate	Isobutyrate	Isovalerate	Valerate	Isocaproate	Caproate
Diets	p=0.406	p=0.697	p= 0.0103	p=0.111	p= 0.159	p= 0.0662	p= 0.923	p= 0.52
BF	15.025±2.23[a]	2.79±2.11[a]	0.17±0.47[a]	0.68±0.04[a]	0.13±0.11[a]	0.07±0.01[a]	0±0[a]	0±0[a]
FF	13.18±3.48[a]	1.48±0.85[a]	0.16±0.19[a]	0.36±0.1[a]	0.04±0.04[a]	0.03±0[a]	0±0[a]	0±0[a]
BF+FF	13.58±1.03[a]	2.45±2.29[a]	0.32±0.72[b]	0.72±0.23[a]	0.57±0.57[a]	0.07±0.07[a]	0.18±0.18[a]	0±0[a]
BF+FD	12.31±0.07[a]	0.52±0.33[a]	0.02±0.03[b]	0.03±0.01[a]	0±0[a]	0±0[a]	0±0[a]	0±0[a]
FF+FD	25.4±0[a]	2.36±0[a]	0±0[c]	5.59±0[a]	0±0[a]	0±0[a]	0±0[a]	0±0[a]
BF+FF +FD	16.57+8.59[a]	0.89±0.65[a]	0±0.08[c]	0.08±0.0[a]	0±0[a]	0±0[a]	0±0[a]	0±0[a]

The results are presented as mean ± deviation. Different letters (a, b, c) indicate significant difference between age groups (P<0.05).

Table 6. Faecal short-chain fatty acids according to age

SCFAs (µmol/g)	Acetate	Propionate	Butyrate	Isobutyrate	Isovalerate	Valerate	Isocaproate	Caproate
Ages (days)	p=0.509	p= 0.26	p= 0.00406	p= 0.743	p= 0.91	p= 0.0562	p= 0.595	p=0.104
0-119	21.20±3.95[a]	3.22±1.67[a]	0.11±0.49[a]	0.65±0.11[a]	0.12±0.12[a]	0.03±0.03[a]	0±0[a]	0±0[a]
120-179	22.05±5.44[a]	3.09±1.21[a]	0.21±0.12[b]	0.76±0[a]	0.26±0[a]	0.25±0[a]	0±0[a]	0±0[a]
180-200	15.13±6.18[a]	1.27±1.035[a]	0.04±0.07[c]	0.61±0.02[a]	0.04±0.015[a]	0±0[a]	0±0[a]	0±0[a]

The results are presented as mean ± deviation. Different letters (a, b, c) indicate significant differences between age groups (P<0.05).

3.6. Correlation between Gut Microbiota Composition and SCFAs

The correlation study between the different SCFAs produced and ASVs in the intestinal flora of infant for the first 200 days of life showed that there is no significant difference at 5% level. The study of the intestinal flora revealed the presence of bacterial families such as, Lachnospiraceae, Ruminococcaceae and Bacteroidaceae able to consume various food fibers from fruits and cereals. Proteins from dairy sources, increased during supplementary feeding. The heatmap of Spearman's correlation coefficients of ASV and SCFAs concentrations measured from infants' faeces during the first 200 days of their life are shown in Figure 1.

Acetate is positively correlated with Bifidobacterium and negatively with Streptococcus and Escherichia-Shigella. Propionate is positively correlated with Bifidobacterium and negatively with Escherichia-Shigella. Similarly, butyrate is positively correlated with Bifidobacterium and negatively with *Escherichia-Shigella*.

3.6. Correlation between the Level of SCFA Production and the Type of Diet with PCA.

The principal component analysis represented by the correlation matrix (Table 7) was established to better define levels of correlation existing between acetate and the different types of SCFAs studied. Correlation matrix analysis indicates that there is a positive correlation between acetate and SCFA like propionate, isobutyrate, butyrate, and isovalerate with respective correlation coefficients of 0.500; 0.307; 0.491; 0.326. There is also a positive correlation between propionate and the other SCFAs which are isobutyrate, butyrate, isovalerate, valerate and isocaproate with respective correlations of 0.661; 0.808; 0.686; 0.460 and 0.339.

There is also a positive correlation between isobutyrate and the other SCFAs which are butyrate; isovalerate; valerate and isocaproate with respective values of 0.645; 0.829; 0.682; 0.468. Similarly, there is a positive correlation between butyrate and the other SCFAs which are isovalerate and valerate with respective values which are 0.747 and 0.545.

However, apart from isobutyrate, there is no link between acetate level and other SCFAs. Isovalerate also correlates positively with acetate, propionate, isobutyrate, butyrate and valerate which correlation coefficients are respectively 0.326; 0.686 ; 0.829 ; 0.747 and 0.772.

The Principal Component analysis carried out shows that the first two axes combine 66.29% of the total variance (the first axis explains 51.87 % and the second axis 14.42 % of this variance) (Figure 2). Axis 1 is strongly correlated on the positive side with the variables caproate, acetate, valerate, butyrate, and isovalerate. This axis expresses pole diets such as LM (Breast milk), breat milk+milk formula. The factor 2 expresses 14.42% of the total inertia of the cloud. It is positively correlated with caproate.

The projection of the different diets on PCA axis1-axis2 factorial plane (Figure 3) indicates that the F1 axis help to discriminate breast milk and artificial milk formula diets. (LM+LA1, LM+LA11; LM+LA2); Breast milk+ milk formula and Food diversification; (LM+LA+DA3; LM+LA+ DA6; LM+LA+DA10; LM+LA+DA5); Breast milk (LM1; LM2; LM4; LM6; LM8) in these positive coordinates. Infant faeces on the above mentioned diets are characterized by high levels of butyrate, acetate, isobutyrate, propionate and valerate production.

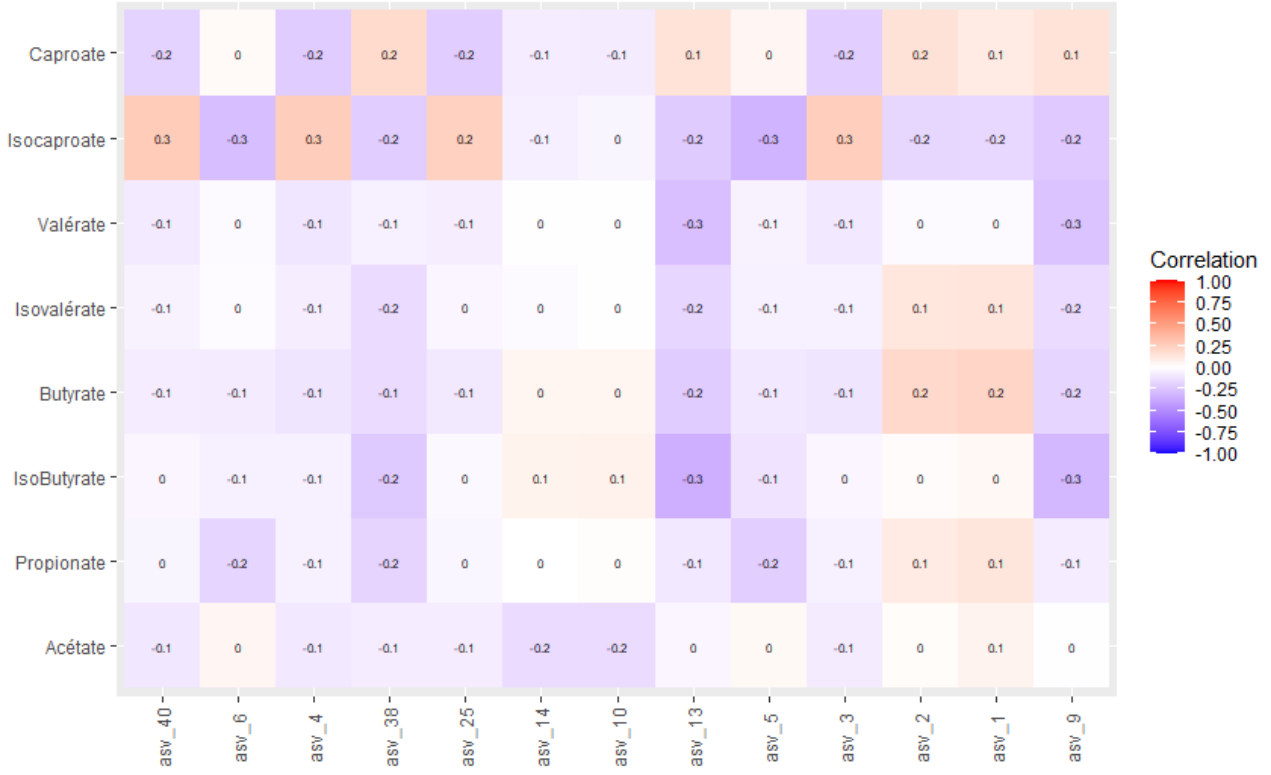

Figure 1. Hierarchical heatmap shows the correlation between SCFAs and ASVs of gut microbiota

Table 7. Correlation matrix between the concentration of SCFAs

Variables	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Isocaproate	Caproate
Actate	1							
Propionate	0.500	1						
IsoButyrate	0.307	0.661	1					
Butyrate	0.491	0.808	0.645	1				
Isovalerate	0.326	0.686	0.829	0.747	1			
Valerate	0.229	0.460	0.682	0.545	0.772	1		
Isocaproate	0.025	0.339	0.468	0.207	0.278	0.148	1	-
Caproate	0.070	0.046	0.101	0.234	0.208	0.277	-0.047	1

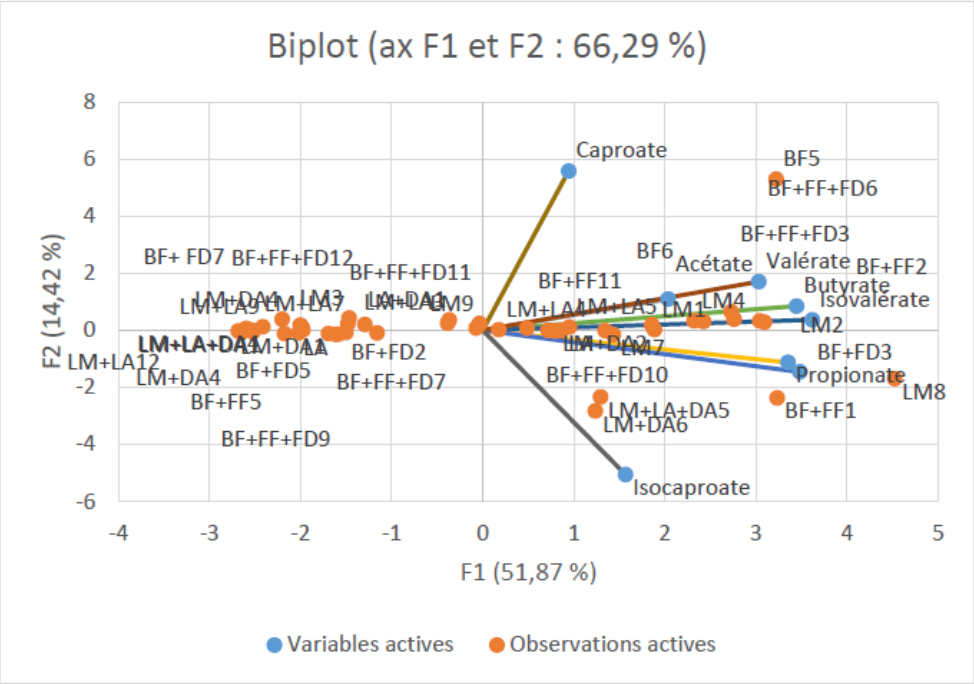

Figure 2. PCA correlation circle performed on different types of SCFAs

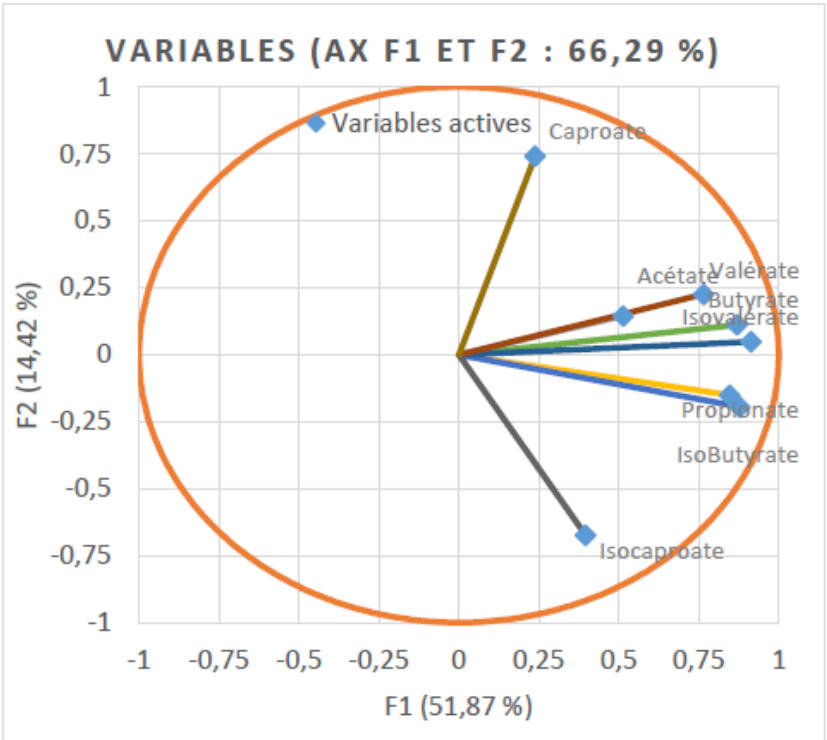

Figure 3. Projection of diets on the PCA F1-F2 factorial plane

4. Discussion

It has been shown that both SCFAs derived from gut microbiota and food diet play physiological effects on host [14,15], while gut microbes have different capabilities to produce and metabolize SCFAs [16,17]. Therefore, gut microbiota with different compositions results in different gut SCFA profiles, which may exert distinct host responses. Infant feeding practices may impact gut microbiota composition and SCFAs production profile.

However, few studies have been carried out on details concerning early introduction of food supplements and the presence of SCFAs in infant faeces.

In this study, faecal SCFA profiles were characterized according to food diet, age, gender and place of residence of the child during his first 200 days of life.

Basically, a part from butyrate, there is no correlation between SCFAs and gender, age, place of residence and food diet.

There is no significant difference between SCFAs (acetate, propionate and butyrate) and gender ($p > 0.05$). However, fecal acetate is more abundant (32.18 ± 14.93µmol/g) in males than in females (32.18 ± 14.93µmol/g). Similarly, propionate is higher (4.74 ± 0.15) in males than in females (3.147 ± 1.60µmol/g). As opposed to acetate and propionate, butyrate is abundant in females than in males. Butyrate is low in both males (0.13 ± 0.3µmol/g) and females (0.28 ± 0.65µmol/g). This difference could be explained by the effect of age, food diet and time of faeces sampling. In addition, the food diet of the breastfeeding mother is able to act on the production profile of SCFAs in the child. SCFAs such as propionate [18], acetate [18] and butyrate [19] have been reported to protect against inflammation of the respiratory tract and this protective effect was attributed to the stimulation of T regs and dendritic cells, capable of preventing TH2 type immune responses [20].

There is no significant difference between the different SCFAs and place of residence ($p > 0.05$). SCFAs are abundant in infants' faeces living in poor towns compared to those living in places with a relatively high standard of living. This difference could be explained by dietary habits and food diet as highlighted by the studies of [19] on both Burkinabe and Italian children. They showed that the diet of Burkinabe children was richer in fiber compared to that of Italian children. People living in less wealthy households do not consume too much processed foods which are rich in fibers. Acetate and propionate are more produced in the district of Abobo than in any other districts.

The acetate found in infants' faeces in Abobo is (34.84 ± 12.26µmol/g), against 21.20 ± 3.95µmol/g in Marcory and 11.49 ± 1.06µmol/g in Cocody. The propionate is 8.55 ± 3.96µmol/g in Abobo, 3.22 ± 1.67µmol/g in Marcory and 4.06 ± 0.67 µmol/g in Cocody.

The abundance of acetate and propionate in faeces is proportional to the practice of exclusive breastfeeding in these districts. However, breast milk contains more carbohydrates which are responsible for acetate production.

HMOs (milk oligosaccharides), known as the main carbohydrates available to the gut microbiota in early life, contain glucose and galactose. They are the main components, with residues of fucose, N -acetylglucosamine and N-acetylneuraminic acid as sub-components. Bifidobacteria metabolise galactose to glucose, and glucose is metabolised to acetate and lactate in a 3: 2 ratio specific to bifidobacteria [21].

Very interesting, but concurring with previous studies [22], Bifidobacterium does not have the metabolic pathway to assimilate fucose, although its abundance was positively associated with intestinal formate concentration in several infants. The production of faecal SCFAs in children according to food diet showed that there was no significant difference between acetate produced in children fed exclusively with milk (BF; FF; BF+FF) compared to children receiving early food supplements (BF+FD; FF+FD and BF+FF+FD) ($p>0.05$). SCFAs are more abundant in infants' faeces starting food supplements early compared to those receiving only milk diet.

These results could be explained by the fact that children receiving supplementary foods have a diverse and complex intestinal microbiota capable of metabolizing food fibers. Acetate appears to be more abundant in infants'faeces fed with LA+DA (25.4 ± 0µmol/g), followed by BF+FF+FD (16.57± 8.59µmol/g) and BF (15.025 ± 2.23µmol/g).

This study shows that acetate is more abundant in infants'faeces receiving more food supplements than those fed with milk diet. The higher concentrations of SCFA (acetate) observed in babies fed with breast milk and food supplements may be due to a variety of bacteria observed in these infants compared to breast-fed babies **[23]** which have therefore, the ability to metabolize substrates in the intestine.

In addition, these results could be explained by the fact that the complementary foods received by these infants and their mothers were richer in carbohydrates propitious to acetate production. It has been shown that exclusively, breastfed infants have higher concentrations of faecal acetate than infants fed with breast milk or mixed diet [24,25,26].

The abundance of acetate in infants' faeces fed with breast milk and food supplements could be linked to a pathology at the intestinal level. Indeed, it has been shown that the production of acetate is linked to protective bifidobacteria which could act to promote the defense functions of host's epithelial cells and protect cells from enteropathogenic infections [27]. Moreover, colonic acetate levels have been shown to be anti-inflammatory through the regulation of colonic regulatory T cells [28]. The data suggest that the composition of faecal SCFAs in most breastfed infants is characterized by a high content of acetic acid. This finding might be associated with protection against diarrhea and respiratory infections in infants [26].

Furthermore, there is no significant difference between the propionate produced in children's faeces consuming milk (BF; FF; BF+FF) and those receiving food supplements (BF+FD; FF+FD and BF+FF+FD) ($p > 0.05$). Propionate and butyrate are more abundant in infants' faeces consuming milk compared to those who started early diet diversification. However, the results show that propionate is more abundant in infant faeces who consume LM diet (2.79 ± 2.11µmol/g), followed by BF+FF diet (2.45 ± 2.29µmol/g) and FF+FD diet (2.36±0µmol/g). Propionate

appears much lower in children fed with BF+FD diet (0.52±0.33). The contribution of the complementary food was a limiting factor for propionate production.

Concerning the results on butyrate production, a significant difference was noticed between butyrate production and BF (0.17±0.47µmol/g); FF (0.16±0.19µmol/g) vs BF+FF (0.32 ± 0.72µmol/g); BF+FD (0.02 ± 0.03µmol/g) vs FF+FD (0±0); BF+FF+FD (0±0.08). Indeed, butyrate is a fermentation product of carbohydrates. Its production is correlated to food diet. The consumption of food supplements reduces the practice of breastfeeding. Likewise, the consumption of cereals leads to iron deficiency or chelation causing a decrease in butyrate production. In this study, the group of infants exclusively fed with breast milk had higher concentrations of propionate and butyrate.

However, the group of infants fed with formula milk or mixed diet, as well as the group of infants receiving early food supplements produced relatively high propionate and butyrate. This production of butyrate could be explained by the presence of some amount of intestinal microbiota and food diet. On the other hand, the results of [29] showed that the group fed with formula milk, had higher concentrations of propionic and butyric acids than breast-fed babies.

Higher concentrations of branched-chain fatty acids, valerate, isobutyrate and isovalerate, derived from amino acid metabolism indicate reduced protein absorption or excessive protein intake due to higher protein content in formula milk compared to breast milk [30].

The results of the analysis of SCFAs production in infant faeces showed that there is a significant difference in the level of butyrate production ($p < 0.05$) and the different age groups. This study reveals that SCFAs are more abundant in infants' faeces with ages ranging fom 4 to 5 months. This finding could be explained by the fact that at this age, they have an intestinal microbiota capable of producing SCFAs.

Thus, butyrate appears to be more abundant in infants' faeces of 4 to 5 months of age (0.21 ± 0.12) than in infants aged 0-119 days (0.11 ± 0.49µmol/g) and 180-200 days (0.04±0.07µmol/g). Compared to propionate which is rather abundant in the age group of 0-119 days. The high production of butyrate is linked to its importance in the body. It reduces the passage of toxins, pollutants and bad bacteria. On the other hand, the results concerning acetate and propionate production show that there is no significant difference between the production of these SCFAs and the different age groups. Acetate and propionate are abundant in infants' faeces aged 0-119 days. The early introduction of food supplements, characterized by a high intake of food fiber and protein, is significantly correlated with the intestinal microbial alpha diversity causing SCFAs production in an established order irrespective of age. The main catabolic end products of food fiber metabolism are the short chain fatty acids (SCFAs) acetate, butyrate and propionate [31].

While acetate is produced in large quantities during infancy from the first three months (e.g., by Bifidobacterium, Lactobacillus and Enterobacteriaceae spp.), butyrate and propionate concentrations are initially very low but increase with infant age [32]. In this study, there is variability in the level of propionate and butyrate due to the early introduction of foods.

Early supplementary feeding (before 3 months of age) has been associated with an increased risk of gastrointestinal and respiratory infections, obesity and allergies, but this may instead be attributed to a short breastfeeding period [33]. These infections and pathologies are due to a low amount of Bifidobacterial community, associated with protection against infectious and immune diseases.

The consumption of cereals as a complementary food [34] revealed the presence of butyrate-producing genera of Lachnospiraceae, while Bacteroides are propionate producers. Bifidobacteriaceae participates in the production of acetate from the beginning of birth. Early food introduction is associated with higher relative abundance of Escherichia-Shigella, Streptococcus and low amount of *Bifidobacterium.*

5. Conclusion

To sum up, exclusive breastfeeding has been associated with a number of beneficial health outcomes in early childhood, including reduced infections, allergic diseases, and improved metabolic markers. Gut microbiota plays an important role along with associated metabolites as a potential causative factor or mediator in the occurrence of subsequent disease states. Breastfeeding is strongly associated with intestinal metabolites, which may be an important mediator in the protective effect of breastfeeding against respiratory infections and diarrhea in infants. The fermentation of fibers by the intestinal microbiota produces short chain fatty acids (SCFAs) which are either absorbed by the intestine or excreted in the faeces.

The dosage of SCFAs showed that they are abundant in infants' faeces starting early food diversification compared to those who received only milk diet.

Children who receive food supplements have a complex and diverse gut microbiota to break down food fibers.

In addition, the results showed that infants from poor districts have an abundant proportion of SCFAs in faeces compared to those living in places with a relatively high standard of living.

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Competing Interests

The authors declare that they have no conflict of interest for this article.

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