Original Article

Reduced crying and favourable stool characteristics in Chinese infants fed milk fat-based formula

Xiao Yang Sheng MD[1], Vanitha Buthmanaban MSc[2], Marlotte Marianne Vonk PhD[2], Anouk Leonie Feitsma PhD[3], Panam Parikh PhD[2]

[1]*Shanghai Institute for Pediatric Research, School of Medicine, Shanghai Jiao Tong University, Shanghai, China*
[2]*FrieslandCampina Development Centre AMEA, Singapore*
[3]*FrieslandCampina, Amersfoort, The Netherlands*

Background and Objectives: Chinese infants consuming four different commercially-available infant formulas were evaluated on gut comfort and stool consistency parameters. **Methods and Study Design:** Gut comfort characteristics were evaluated during a 7-day cross-sectional observational study in 409 healthy, term, exclusively formula-fed infants via questionnaires and fecal parameters. **Results:** The stool consistency and color scores were different between the infants consuming one of the four commercially-available infant formulas including different fat sources, i.e. one milk fat-based (IF1), two structured vegetable fat blend-based (IF2 and IF4) and one palm oil-free vegetable fat blend-based (IF3). The scoring pattern showed more 'soft-formed' stools for IF1-consuming infants compared to infants consuming IF2, IF3 or IF4. In addition, a lower amount of green feces was observed in combination with an increase in golden-colored feces for IF1-consuming infants compared to the other groups. Furthermore, IF1-consuming infants reported less fussy/crying time during the night and less gut discomfort. Infants consuming milk fat-based IF1 showed significantly lower fatty acid soaps compared to palm-oil free IF3-fed infants. **Conclusions:** Infants consuming milk fat-based IF1 experienced less gut discomfort compared to infants consuming other commercially-available infant formula. Lower fecal fatty acid soap levels, fussy/crying time during the night and gut discomfort were observed. These findings contribute to the current understanding of the association between lipid structure and gut comfort parameters. However, the suggested benefits noted cannot be fully linked to the effect of fat blend differences since formulas differ in ingredient-sourcing and processing. Future research should confirm the added benefit of milk fat-based infant formulas to improve gut comfort parameters.

Key Words: crying, stool consistency, stool color, infant, milk fat, gut comfort

INTRODUCTION

For an infant, human milk is the preferred nutrition (EU Directive 2006/141), but when this is not possible, infant formula is the most suitable alternative. A wide range of infant formulas are available on the market, varying in the sourcing and processing of their ingredients, e.g. the fat blend. Fat is an important nutrient providing the infant with energy and building blocks required for healthy growth and development. Besides the energy aspect, fat and proteins are considered important in regulating the infants' gut comfort. The majority of infants suffers from gastro-intestinal complaints, albeit to a different extent.[1-3] Gut comfort is determined by a complex combination of several factors: the ingredient composition, digestibility of nutrients, production of metabolites, and interaction of nutrients/metabolites with the epithelial barrier can influence gut comfort parameters.[4,5]

Fat is one of the main components present in infant formula and consists for about 98% of triglycerides (TG). TG are composed of a glycerol backbone with three fatty acids attached to three different positions called sn-1, sn-2 and sn-3. TG present in human milk fat are composed of nearly 200 different fatty acids[6,7] with different length (ranging from C4:0-C26:0) and saturation level. Specific fatty acids have their favorable sn-position at the glycerol backbone. About 40% of the fatty acids in human milk are represented by the long-chain saturated fatty acids (LCSFA), which are defined as saturated fatty acids with twelve or more carbon atoms (lauric acid). From all LCSFA in human milk, palmitic acid accounts for half of the LCSFA (~22%).[8] From all palmitic acid, 70-88% is attached at the sn-2 position.[9-12] In contrast, unsaturated fatty acids (UFA) are predominantly attached to the sn-1 and sn-3 positions of the TG.[9,10] Fat blends used in infant formula are derived from several sources: vegetable oils (e.g. palm oil, rapeseed oil, sunflower oil, safflower oil,

Corresponding Author: Dr Anouk L Feitsma, FrieslandCampina, Amersfoort, The Netherlands.
Tel: +31 646911327
Email: Anouk.Feitsma@frieslandcampina.com
Manuscript received 25 April 2019. Initial review completed 23 July 2019. Revision accepted 05 November 2019.
doi: 10.6133/apjcn.202003_29(1).0019

corn oil, soybean oil and coconut oil), structured vegetable fat (OPO, InFat), and bovine milk fat (either as anhydrous milk fat or cream). These fat sources differ in their overall fatty acid- and TG composition with a different percentage of palmitic acid at the sn-2 position. The possible health aspects related to specific fat blends and the positioning of palmitic acid used in infant formulas are described in literature.[8,13] This positioning of the fatty acids on the backbone of the TG influences the digestion and absorption of the fatty acids.[14-16] In particular, lipases in the gastro-intestinal tract (gastric and pancreatic lipase) specifically hydrolyze the fatty acids positioned at sn-1 and sn-3, while the sn-2 fatty acid remains on the glycerol backbone as sn-2-monoglyceride. This is of special importance for LCSFA, as they are not well absorbed as free fatty acids and are more efficiently absorbed in the intestine as sn-2-monoglycerides.[13] As an example, it has been described that the amount of palmitic acid, a LCSFA, positioned at sn-2 might be linked to gut comfort in infants.[17-21] In particular, free palmitic acid, like other LCSFA, is able to form indigestible complexes with calcium in the gut lumen (i.e. soap formation).[20,21] These fatty acid soaps are not absorbed and are excreted in feces, thereby negatively affecting the absorption of energy (fat) and minerals (calcium).[21] Studies have also reported that infants with increased levels of fatty acid soaps in their feces suffer from increased gastro-intestinal discomfort.[17-21]

Although the link between gut comfort and fatty acid soaps has been made multiple times, it is challenging to assess gut comfort in a young infant. Several questionnaires are available and validated to assess different gut comfort parameters. These questionnaires assess stool characteristics (Amsterdam Infant Stool Scale (AISS) or Bristol Stool Chart), crying or fussiness behavior and gastro-intestinal complaints. In China, theories of Traditional Chinese Medicine (TCM) greatly influence the dietary habits and lifestyle practices of its people. Health is seen as harmony between yin and yang and this balance must be maintained for well-being and to keep illness at bay. Heatiness is of particular concern of mothers with infants. Mothers believe if their infant is heaty, the ability to digest and absorb food well is lowered and thereby potentially affecting the child's growth and development. Moreover, the stagnation of "qi" due to unabsorbed food can lead to constipation. Other manifestations of 'heatiness' are the presence of symptoms such as dry feces, sleeping problems, eye boogers, palm temperature, dry cough, bleeding nose, and dry mouth. Heatiness was therefore assessed in this survey using the 'Clinical manifestations of infants and young children heatiness questionnaire', a tool developed together with a TCM practitioner for this study.

The aim of the current study was to identify differences in stool characteristics and gut comfort parameters between four commercially-available infant formulas, in China.

METHODS

The current study was a cross-sectional observational study of healthy, term, exclusively formula-fed infants consuming one of four different commercially-available infant formulas on the Chinese market. The study outline is depicted in Figure 1.

Study design and population

Included infants were healthy, term (gestational age 37-42 weeks and birth weight 2.5-4.0 kg), aged 0-4 months and exclusively formula-fed (consuming a commercially-available formula) without weaning food intake. All infants were exclusively fed with the formula of their group for at least 1 week prior to the study. The composition of the different formulas is listed in Table 1. IF1 contained milk fat combined with vegetable fat, IF2 and IF4 contained structured vegetable fats and IF3 contained a palm oil-free fat blend. The exclusion criteria were: breastfeeding (partial and full) <3 weeks prior to the study, complementary feeding, congenital condition and/or illness (previous/current) that could interfere with the study, cow's milk allergy (CMA) or at-risk for CMA, lactose intolerance, use of antibiotics, use of Western or traditional Chinese medicine for gastro-intestinal symptoms and appetite changes and use of probiotic supplements <2 weeks prior to the study. Screening and enrollment was performed between birth and 120±2 days of age. Parents and/or caregivers were asked to fill in daily questionnaires on stool characteristics (AISS), gastro-intestinal symptoms (subject diary and questionnaire on

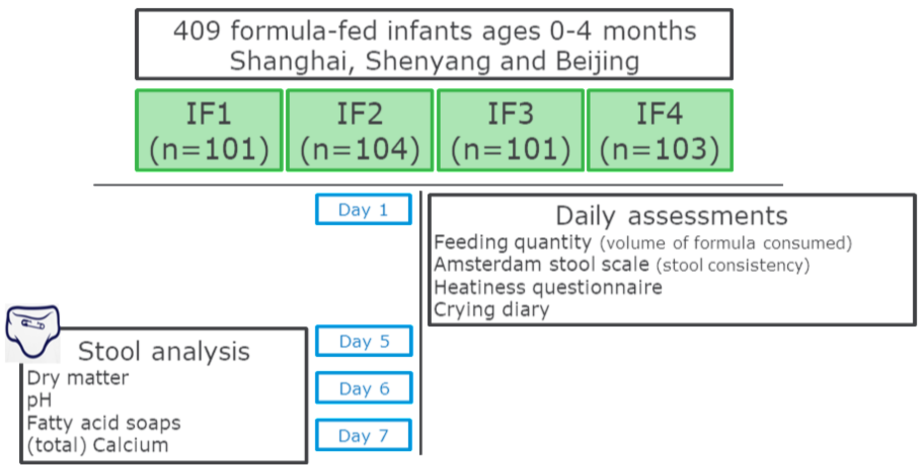

Figure 1. Study outline.

Table 1. Infant formula characteristics†

Formula	IF1 (n=101)	IF2 (n=104)	IF3 (n=101)	IF4 (n=103)
Energy (kJ)	2130	2122	2119	2076
Protein (g)	11.0	10.2	11.3	11.0
Fat (g)	27.0	27.3	27.4	25.0
LCSFA (mol%)	44.6	38.9	34.2	39.4
C16:0 (wt%)	25.0	23.4	8.5	19.4
C16:0 sn-2 (% total C16:0)	19.9	43.5	10.2	40.1
Ca+Mg (mmol)	9.9+2.0	8.1+1.5	9.6+2.1	7.8+1.3
Prebiotic fibers	1.9	2.3	3.1	3.0

IF: infant formula; LCSFA: long-chain saturated fatty acids; Ca+Mg: Calcium + Magnesium
†Levels present in the different infant formulas per 100 g powder.

infant/toddler GI symptoms), and subject diaries for crying/fussiness, timing and amount of feedings and the clinical manifestations of infant, and young children heatiness questionnaire for 7 consecutive days. Parents and/or caregivers were asked to assign a score on a scale of 100 for individual heatiness symptoms. Infants were categorized as no heatiness, mild heatiness, moderate heatiness and severe heatiness based on the total (0-20, 20-50, 50-80 and 80-100, respectively). Parents and/or caregivers also scored the stool of every defecation with the infant stool form scale for all seven days and data were recorded in the subject diary. Furthermore, parents and/or caregivers were asked to collect feces samples during the final three days of the study.

This study was approved by the IRB of the Shanghai Nutrition Society (Ethical Review [2016] No.005) and therefore is performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (October 1996 amendment). Parents or legal guardians gave written, informed consent prior to the inclusion in the study.

Feces collection
Stool samples were collected 3 days prior to the collection and evaluation visit using stool collection kits (Taizhou xingke medical supplies co. LTD, Taizhou, China) provided to the parents and/or caregivers. Each day, feces was transferred from the diaper into feces testing cups. Collected samples were stored and transported to the laboratory at -20 °C.

Fecal analyses
Fecal samples from the two testing cups collected in the 3 days prior to the visit were pooled and analyzed for pH (in fecal water), dry matter (air dried in oven at 103 ˚C) and crude fat (extraction via method of Weibull-Berntrop). Calcium was extracted from the feces samples with 24% trichloro-acetic acid. ICP-MS inductively coupled plasma mass spectrometry (Inorganic Ventures, Christiansburg, USA) was used to determine the calcium content. Fatty acids were analyzed by GC-FID (Sigma, Osterode am Harz, Germany) after Folch extraction and fatty acid methyl ester derivatization. The soap and non-soap fatty acids were determined according to the method of Quinlan et al.[21]

Statistical analysis
Based on power calculation, the study aimed to have 400 participants completing the study. To cover a drop-out rate of 10%, the aimed amount of participants to enroll was 440. Data evaluation was performed per protocol analysis using SAS version 9.3 (SAS Institute Inc., Cary, USA).

Differences in baseline characteristics were analyzed using the analysis of variance (ANOVA) for age, birth weight, birth height, head circumference, gestational weeks, Apgar score, enrolment weight and enrolment height, Kruskal-Wallis for delivery type and socio-economic status parameters and Chi-square for gender. All statistical analyses were performed with a significance level of 0.05.

The daily amount of product consumption was calculated by summing up the start amount at each feeding process and subtracting the remaining amount at the end of each feeding process. These data were analyzed using repeated measure ANOVA. Stool consistency and stool color parameters were assessed with type 3 GEE analysis for repeated measured (at each defecation on each of day 1 to day 7) categorical data was used to evaluate product differences adjusted for covariates (study site, day, defecation number, gender, daily formula feeding amount, delivery type and subject age). Stool consistency (1=watery; 2=soft; 3=formed; 4=hard), stool amount (1=smear; 2=up to 25%; 3=25-50%; 4=>50%) and stool color (1 to 6 from light to dark) were evaluated at each defecation. The daily average score of these characteristics was calculated for each subject as the mean of all the scores observed from that subject on each day from Day 1 to Day 7. Evaluation of product difference was performed on the scores for each defecation (multinomial variable), not on the daily average scores. Bonferroni adjustment was applied for multiple comparison among study products.

Crying log was recorded at every 15 minutes during the four periods of a day (morning, afternoon, evening and night) for the following status of study subjects: sleeping; awake, satisfied; awake, fussy; and awake, crying. The number of time boxes (15 minutes) that study subjects were fussy or crying was counted for each period. The total time of fussy and crying during each period was then calculated as the number of boxes times 15 minutes. Fussy and crying time was summarized using mean and standard deviation, and evaluated using a repeated-measure ANOVA. The total score of heatiness symptoms (maximum score is 100) were calculated as the sum of the individual scores for all heatiness symptoms. Heatiness is defined according to the total heatiness symptom score: 0-20 no heatiness; 20-50 mild heatiness; 50-80 moderate heatiness; 80-100 severe heatiness.

Pairwise product comparison was performed for the (non-)soap fatty acids levels with odds ratio (OR) of an outcome levels having higher ordered values for one product versus the other. Bonferroni adjustment was applied for multiple comparison among study products.

Correlation analyses were performed by Spearman's rho test taking along all participants.

RESULTS

Table 2 displays the summary of subjects' baseline characteristics by study group. All baseline characteristics were distributed similarly in each product group. In total 1348 infants were screened from which 433 infants were enrolled in the study. Final number of infants completing the survey was 409.

In the daily feeding frequency and amount, no differences were observed between the groups (Table 3). The feeding frequency was around 6 times a day and mean intake was 731±96 mL per day for all the groups.

The outcome of the questionnaires on the AISS, GI comfort and heatiness are depicted in Table 3. For the stool characteristics from the AISS, no significant differences were observed in daily stool frequency and stool amount. The stool consistency of the first defecation of each day was significantly different between the children consuming IF1 vs IF2, IF3 and IF4 (all $p<0.0001$) but no significant difference between children consuming IF2, IF3 and IF4 were observed (Table 3). It could be observed that more soft/formed stools were reported in the milk fat-based IF1 group. The scores A and D (watery and hard) were less reported for children consuming IF1 in comparison to all other groups (IF2, IF3 and IF4). Within the heatiness questionnaire IF1-fed infants scored significantly lower on "dry feces/yellow urine" compared to the other groups (Table 3).

For the stool color (Table 3), children consuming the milk fat-based IF1 had significantly different stool color scores compared to children consuming IF2 ($p=0.001$), IF3 ($p<0.0001$) and IF4 ($p=0.0003$), while no significant differences were observed between the IF2, IF3 and IF4 groups. A higher percentage of IF1-fed infants reported score I, which means golden colored stool and less green stool (score III).

The analysis of gastro-intestinal symptoms (Table 3) showed that a significant difference was observed for abdominal distension ($p=0.001$), burping ($p=0.004$), flatulence ($p=0.0001$), diarrhea and constipation ($p<0.0001$) (Table 3). Further analyses showed that the milk fat-based IF1 is significantly scoring lower from IF2 ($p=<0.0001$ for abdominal distension, burping, flatulence, diarrhea and constipation), IF3 (p=<0.0001 for abdominal distension, flatulence, diarrhea and constipation, $p=0.0008$ for burping) and IF4 ($p=<0.0001$ for abdominal distension, flatulence, diarrhea and constipation, $p=0.002$ for burping), but IF2, IF3 and IF4 are not different from each other.

Fussy and crying time duration was evaluated in minutes during the morning, afternoon, evening and night (Figure 2). A significant product difference was observed for the fussy and crying time during the night ($p=0.0003$) while the other moments of the days were not different between the groups. Infants fed with IF1 had significantly shorter fussy and crying time during the night compared to the subjects consuming IF2 ($p<0.0001$), IF3 ($p=0.0007$) or IF4 ($p=0.004$), while no difference was noted between other groups. Mean fussy and crying time during the night per group were 26.5±20.3, 38.9±24.9, 36.7±27.1 and 37.4±28.4 respectively. This was also reflected in a lower score on sleeping problems in the heatiness questionnaire for children fed with the milk fat-based IF1 compared to the other groups (Table 3, $p<0.0001$).

A spearman's rho test was performed to check for the correlation between all parameters measured in the diaries, showing a correlation between stool consistency score and stool amount ($\rho=0.26$; $p=0.001$), stool color ($\rho=0.16$; $p=0.001$) and a mild negative correlation with GI symptoms of abdominal distension, burping, flatulence and diarrhea (ρ all ~-0.10 with $p=0.001$). Several GI symptoms (abdominal distension, burping, flatulence, diarrhea, colic, diaper dermatitis and back arching) correlate with each other (ρ ~0.2-0.3 with $p=0.001$) indicating that the GI symptoms co-occur when present.

An overview of the fecal measurements is depicted in Table 4. The fecal fatty acid soaps are significantly different across all groups ($p=0.002$). Pair-wise analyses showed a significant lower level of fatty acid soaps for IF1-fed infants compared to infants fed with IF3 ($p=0.003$) (Figure 3), and a trend for significance both between IF1- and IF4-fed infants ($p=0.06$) and IF2- and IF3-fed infants ($p=0.06$).

Table 2. Baseline characteristics[†]

Infant characteristics	IF1 (n=101)	IF2 (n=104)	IF3 (n=101)	IF4 (n=103)	*p*-value
Gestational age (wk)	39.3±1.2	38.9±1.2	39.3±1.2	39.1±1.3	0.115
Age (days)	93.9±21.6	100.7±20.6	96.5±21.9	100.0±21.3	0.080
Enrollment Weight (kg)	6.0±0.9	6.3±0.8	6.1±0.8	6.2±0.9	0.168
Enrollment height (cm)	60.1±4.3	61.2±3.2	60.7±3.5	60.9±3.6	0.199
Gender (% male)	39.6	43.3	40.6	52.4	0.228
Delivery (% vaginal)	61.4	57.7	68.3	68.0	0.308
Maternal Characteristics					
Education (% completed college)	45.5	52.9	51.5	53.4	0.123
Occupation (%)	93.1	92.3	90.1	91.3	0.882

[†]Baseline characteristics of the infants included in each of the study groups and their mothers. Group means±SD and the *p*-value of the overall group difference.

Table 3. Questionnaire outcomes †

	IF1	IF2	IF3	IF4	*p*-value
Daily feeding amount (mL)	725±90	737±99	727±99	735±95	0.340
Daily feeding frequency (#)	5.9±0.9	5.9±0.9	5.8±0.9	5.7±0.8	0.312
Amsterdam Stool Scale‡					
Daily stool frequency (#)	1.5±0.8	1.5±0.9	1.5±0.9	1.3±0.5	0.300
Stool consistency first defecation (mean score)	2.5±0.6	2.2±0.8	2.2±0.8	2.2±0.8	<0.0001
Stool consistency # reports/day (mean 7 days)					
No stool	2.1	3.6	3.6	4.7	
Watery stool	4.6	16.6	13.4	12.1	
Soft/formed stool	91.1	74.0	74.7	76.4	
Hard stool	3.1	9.9	9.3	9.7	
Stool amount of the first defecation (mean score)	2.3±0.8	2.5±0.9	2.4±0.8	2.2±0.8	0.117
Stool color of the first defecation (mean score)	2.2±1.1	2.5±0.9	2.6±1.0	2.4±0.8	0.0008
Stool color # reports/day (mean 7 days)					
No stool	2.1	3.6	3.6	4.7	
I (golden)	24.9	12.7	7.9	17.0	
II (orange)	46.3	40.7	41.4	28.1	
III (green)	20.9	39.3	37.1	50.0	
IV (brown)	2.4	4.7	5.9	2.4	
V (grey/black)	1.3	1.4	2.3	0.6	
VI (white)	3.1	1.6	2.9	0.1	
GI symptoms questionnaire (mean score 7 days)					
Abdominal distension	1.07±0.7	1.38±0.8	1.38±0.7	1.31±0.6	0.001
Burping	1.42±1.1	1.78±0.9	1.67±0.9	1.66±0.9	0.004
Flatulence	1.08±0.8	1.41±0.8	1.31±0.6	1.26±0.6	0.0001
Diarrhea	0.82±0.6	1.09±0.6	1.07±0.5	1.11±0.5	<0.0001
Constipation	0.87±0.7	1.13±0.6	1.16±0.6	1.13±0.5	<0.0001
Colic	1.20±0.5	1.15±0.6	1.16±0.4	1.15±0.4	0.167
Diaper dermatitis	1.13±0.4	1.12±0.5	1.17±0.5	1.14±0.5	0.286
Back arching	1.16±0.5	1.20±0.5	1.16±0.4	1.21±0.5	0.661
Vomiting (#)	0.25±0.71	0.29±0.82	0.21±0.54	0.28±0.88	0.935
Regurgitation (#)	0.12±0.55	0.09±0.44	0.12±0.48	0.09±0.38	0.820
Heatiness questionnaire (day 1)					
Dry feces, yellow urine (% normal stool frequency)	93	66	68	62	<0.0001*
Aphtha and dry mouth (% none)	98	96	98	99	0.129*
Sleeping problem (% sleep normally)	90	54	55	59	<0.0001*
Eye boogers (% normal)	92	90	93	93	0.123*
Dry skin (% normal)	97	91	90	92	0.636*
Bleeding nose (% none)	100	98	100	100	NA
Dry cough (% none)	93	94	91	95	0.188*
Throat pain (% none)	100	98	100	99	0.677*
Palm temperature (% cool and wet)	90	91	86	88	0.645*
Anus color (% pink)	90	92	94	92	0.565*
Bad breath (% none)	89	95	96	95	0.233*
Overall heatiness level (% No heatiness)	100	94	97	96	0.317*

†Cells indicate the mean score±SD reported over 7 days in each treatment group for the parameters analyzed from the GI symptoms.
‡Amsterdam stool scale and heatiness questionnaires. For stool consistency and stool color the # of participants reporting each individual score over 7 days is indicated per group.
The *p*-value column shows the result of the overall product comparison. **p*-value on mean score per question for product effect.

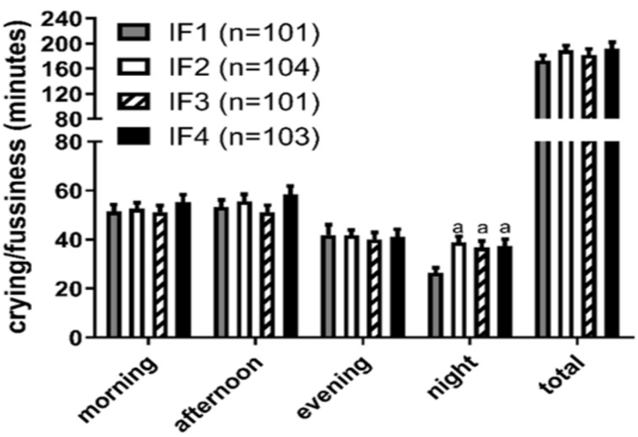

Figure 2. Minutes of crying and/or fussiness reported in the diaries for infants fed with IF1, IF2, IF3 or IF4. Mean and SEM of 7 days questionnaires. The 'a' indicates a significant difference from IF1 ($p \leq 0.004$).

Table 4. Stool parameters†

Parameter	IF1	IF2	IF3	IF4	p-value
pH	5.15 (0.38)	5.26 (0.42)	5.27 (0.42)	5.17 (0.45)	0.10
Calcium (mg/100 g)	514 (217)	510 (305)	561 (328)	590 (335)	0.16
Fatty acids total (mg/g dry stool)	1.08 (0.74)	1.18 (0.45)	1.29 (0.63)	1.21 (0.51)	0.096
Soap fatty acids	0.49 (0.29)	0.53 (0.30)	0.67 (0.46)	0.62 (0.38)	0.002*
Non-soap fatty acids	0.59 (0.68)	0.65 (0.33)	0.62 (0.40)	0.59 (0.31)	0.76
Dry matter (%)	19.9 (4.1)	20.3 (3.1)	20.7 (3.1)	20.9 (3.9)	0.19

†Cells indicate the mean value (SD) measured in the pooled stool samples collected on day 5, 6 and 7 in each treatment group for the indicated parameters.
The p-value column shows the result of the overall product comparison. *Significant difference across groups.

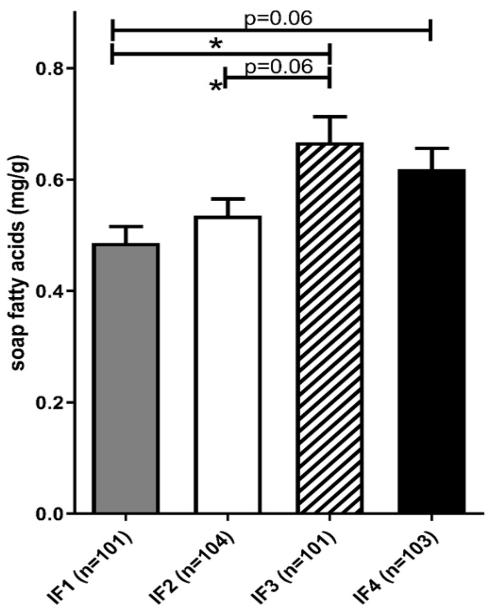

Figure 3. Fatty acid soap levels (mg/g) in dry feces. **p=0.003. IF: infant formula

For pH, a statistical trend for differences between the groups was observed (p=0.10). Therefore additional group by group comparisons were performed, showing a small difference in fecal pH between the infants fed with the milk fat-based IF1 compared to infants fed with IF3 (p=0.04). A Spearman's rho test for correlation, taking along all participants, showed a small, but significant correlation between soap fatty acids and fecal pH (ρ=0.13; p=0.001). This indicates that more fatty acid soaps were present in the fecal sample with higher pH values, independent of the study group (data not shown).

DISCUSSION

The current study assessed and compared the stool characteristics and gut comfort parameters between infants exclusively formula-fed with one of four commercially-available infant formulas, in a cross-sectional observational study in China. Infants fed with IF1 (milk fat-based formula) showed different stool consistency scores (more soft/formed stools and less watery and hard stools) and stool color (more golden color and less green color) compared to infants fed with IF2, IF3 or IF4. Furthermore, infants fed with IF1 reported less crying/fussy time during the night and less GI symptoms (flatulence, burping, diarrhea, abdominal distension and constipation). Within the fecal analysis, a lower amount of fecal fatty acid soaps was observed for the infants fed with the milk fat-based IF1 compared to infants fed with IF3.

The majority of infants suffers from gastro-intestinal complaints, albeit to a different extent.[1–3] Den Hertog et al. described that the feces of breastfed children at 3 months of age is a mix of soft/formed stools (~21%) and watery (79%) while formula-fed infants report 72% soft/formed stools and 27% watery stools.[22] Although the children in the current study are on average a bit older than 3 months (~100 days), they do report comparable numbers of soft/formed stools compared to the formula fed infants in the study indicating that both study populations have a similar distribution. For stool color, 77% of the breastfed children are reported to have yellow feces and 23% green feces compared to respectively 44% and 54% in the formula-fed group.[22] Although the scoring of the color is via a 4-point scale instead of the 7-point scale used in the current study, in the current study 70% of the children in the milk fat-based IF1 group are reported with golden/orange colored feces and 21% with green feces. Children in the IF2, IF3 and IF4 groups were reported to have 44-51% golden/orange feces and 37-49% green feces. These data imply that the feces color for the IF2-, IF3- and IF4-fed infants is similar to what is described in literature and the fecal color of children fed with IF1 reflects the observations from breastfed infants.[22]

One of the main differences, in the composition of the infant formulas included in the current cross-sectional observational study is the fat blend used. IF1 includes milk fat, IF2 and IF4 contain structured vegetable fat and IF3 contains a palm oil-free fat blend. To our knowledge, the current study is the first study assessing gut comfort characteristics upon consumption of infant formula containing milk fat compared to infant formulas with several (structured) vegetable fat sources in infants. Studies in literature assessing the effect of fat source on gut comfort and stool characteristics rather focus on the TG structure of the fat source included. For example, infants fed with infant formulas containing fat blends with increased levels of palmitic acid at the sn-2 position report lower amount of gut discomfort and lower fecal fatty acid soap levels are found.[18-20,23] The current study shows that infants fed with a milk fat-containing formula (IF1) have lower fecal fatty acid soaps compared to infants fed with a formula including a palm oil-free fat blend (IF3) although the amount of palmitic acid present in the formula is much higher. It has been described in several publications that palmitic acid is a major fatty acid present in fatty acid soaps, but other LCSFA including lauric acid, myristic acid, stearic acid and also the unsaturated fatty acids oleic acid and linoleic acid are present in the fatty acid soaps.[20,21,23] Since no specific fatty acids are analyzed in the feces samples (both for the soap and non-soap fatty acids) in the current study, only hypotheses can be postulated. Also the trend for lower levels of fatty acid soaps observed for infants consuming milk fat-based formula (IF1) compared to infants fed with structured vegetable fat (IF4) and infants fed with structured vegetable fat-formula (IF2) vs palm oil-free formula (IF3) indicate towards this. Probably, palmitic acid in total TG composition and at the sn-2 position is not the only parameter resulting in increased or reduced fatty acid soaps. More research is required to depict the differentiating factors in the fatty acid composition on fatty acid soap formation.

Clear differences in the outcome parameters on stool characteristics and gut comfort were observed in the current study. The significant correlation between several GI symptom parameters from the questionnaires shows that the assessed parameters have an internal consistency. This endorses the quality of the study data collected and the identified effects. Furthermore, to our knowledge, this is the first study assessing both questionnaires frequently used in European gut comfort studies (AISS, crying diary) and a Heatiness questionnaire developed for this study but based on a frequently used concept used in Asian populations. Effects observed on stool consistency are reflected in both types of questionnaires, which strengthens the reliability of the questionnaires included.

The current study design (cross-sectional observational study) allows showing differences and associations but no causal relation. To assess a causal relation, a randomized controlled intervention trial is required. Since four different commercially-available infant formulas from different manufacturers were taken along in the current study, all formulas vary in the processing and sourcing of ingredients used (fat, protein, minerals, vitamins, oligosaccharides). Therefore, identified effects cannot be attributed to one (group) of the ingredients. Nevertheless, the current study confirmed that it is worthwhile to further explore the health benefits that can be accomplished with bovine milk fat in infant formula.

ACKNOWLEDGEMENTS

We thank the SPRIM China team, Charlie Zhang MD and Cathy Sun, for study operations.

AUTHOR DISCLOSURES

Declaration of interest: VB, MMV, PP and ALF are co-workers of FrieslandCampina. The study is sponsored by FrieslandCampina.

REFERENCES

1. Iacono G, Merolla R, D'Amico D, Bonci E, Cavataio F, Di Prima L, Scalici G, Indinnimeo L, Averna MR, Caroccio A. Gastrointestinal symptoms in infancy: a population-based prospective study. Dig Liver Dis. 2005;37:432-8. doi: 10.1016/j.dld.2005.01.009.
2. Mugie SM, Benninga MA, Di Lorenzo C. Epidemiology of constipation in children and adults: A systematic review. Best Pract Res Clin Gastroenterol. 2011;25:3-18. doi: 10.1016/j.bpg.2010.12.010.
3. Benninga MA, Nurko S, Faure C, Hyman PE, St. James Roberts I, Schechter NL. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology. 2016;150:1443-55.e2. doi: 10.1053/j.gastro.2016.02.016.
4. Tunc VT, Camurdan AD, Ilhan MN, Sahin F, Beyazova U. Factors associated with defecation patterns in 0-24-month-old children. Eur J Pediatr. 2008;167:1357-62. doi: 10.1007/s00431-008-0669-2
5. Weaver LT, Desai M, Austin S, Arthur HML, Lucas A, Hales CN. Effects of protein restriction in early life on growth and function of the gastrointestinal tract of the rat. J Pediatr Gastroenterol Nutr. 1998;27:553-9. doi: 10.1097/00005176-199811000-00012.
6. Jensen RG, Ferris AM, Lammi-Keefe CJ, Henderson RA. Lipids of bovine and human milks: a comparison. J Dairy Sci. 1990;73:223-40. doi: 10.3168/jds.S0022-0302(90)78666-3.
7. Månsson HL. Fatty acids in bovine milk fat. Food Nutr Res. 2008;52:1-3. doi: 10.3402/fnr.v52i0.1821.
8. Hageman JHJ, Danielsen M, Nieuwenhuizen AG, Feitsma AL, Dalsgaard TK. Comparison of bovine milk fat and vegetable fat for infant formula: Implications for infant health. Int Dairy J. 2019;92:37-49. doi: 10.1016/j.idairyj.2019.01.005.
9. Bracco U. Effect structure on fat absorption. Am J Clin Nutr. 1994;60:1002S-9S. doi: 10.1093/ajcn/60.6.1002S.
10. Straarup EM, Lauritzen L, Faerk J, Høy C-E, Michaelsen KF. The stereospecific triacylglycerol structures and Fatty Acid profiles of human milk and infant formulas. J Pediatr Gastroenterol Nutr. 2006;42:293-9. doi: 10.1097/01.mpg.0000214155.51036.4f.
11. López-López A, López-Sabater MC, Campoy-Folgoso C, Rivero-Urgell M, Castellote-Bargalló AI. Fatty acid and sn-2 fatty acid composition in human milk from Granada (Spain) and in infant formulas. Eur J Clin Nutr. 2002;56:1242-54. doi: 10.1038/sj.ejcn.1601470.
12. Sun C, Wei W, Su H, Zou X, Wang X. Evaluation of sn -2 fatty acid composition in commercial infant formulas on the Chinese market: A comparative study based on fat source and stage. Food Chem. 2018;242:29-36. doi: 10.1016/j.foodchem.2017.09.005.
13. Innis SM. Dietary triacylglycerol structure and its role in infant nutrition. Adv Nutr An Int Rev J. 2011;2:275-83. doi: 10.3945/an.111.000448.
14. Lindquist S, Hernell O. Lipid digestion and absorption in early life: an update. Curr Opin Clin Nutr Metab Care. 2010;13:314-20. doi: 10.1097/MCO.0b013e328337bbf0.
15. Jensen RG, deJong FA, Lambert-Davis LG, Hamosh M. Fatty acid and positional selectivities of gastric lipase from premature human infants:in vitro studies. Lipids. 1994;29:433-5. doi: 10.1007/BF02537313.
16. Rogalska E, Ransac S, Verger R. Stereoselectivity of Lipases. Biochemistry. 1990;265:20271-6.
17. Petit V, Sandoz L, Garcia-Rodenas CL. Importance of the regiospecific distribution of long-chain saturated fatty acids on gut comfort, fat and calcium absorption in infants. Prostaglandins Leukot Essent Fat Acids. 2017;121:40-51. doi: 10.1016/j.plefa.2017.05.007.
18. Kennedy K, Fewtrell MS, Morley R, Abbott R, Quinlan PT, Wells JCK, Bindels JG, Lucas A. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: Effects on stool biochemistry, stool characteristics, and bone mineralization. Am J Clin Nutr. 1999;70:920-7. doi: 10.1093/ajcn/70.5.920.
19. Nowacki J, Lee H-C, Lien R, Cheng S-W, Li S-T, Yao M, Northington R, Jan I, Mutung G. Stool fatty acid soaps, stool consistency and gastrointestinal tolerance in term infants fed

infant formulas containing high sn-2 palmitate with or without oligofructose: a double-blind, randomized clinical trial. Nutr J. 2014;13:105. doi: 10.1186/1475-2891-13-105.
20. Yao M, Lien EL, Capeding MRZ, Fitzgerald M, Ramanujam K, Yuhas R, Northington R, Lebumfacil J, Wang L, DeRusso PA. Effects of term infant formulas containing high sn-2 palmitate with and without oligofructose on stool composition, stool characteristics, and bifidogenicity. J Pediatr Gastroenterol Nutr. 2014;59:440-8. doi: 10.1097/MPG.0000000000000443
21. Quinlan PT, Lockton S, Irwin J, Lucas AL. The relationship between stool hardness and stool composition in breast- and formula-fed infants. J Pediatr Gastroenterol Nutr. 1995;20:81-90. doi: 10.1097/00005176-199501000-00014.
22. den Hertog J, van Leengoed E, Kolk F, van den Broek L, Kramer E, Bakker E-J, Bakker-van Gijssel E, Bulk A, Kneepkens F, Benninga MA. The defecation pattern of healthy term infants up to the age of 3 months. Arch Dis Child - Fetal Neonatal Ed. 2012;97:F465-70. doi: 10.1136/archdischild-2011-300539
23. Béghin L, Marchandise X, Lien E, Bricout M, Bernet J-P, Lienhardt J-F et al. Growth, stool consistency and bone mineral content in healthy term infants fed sn -2-palmitate-enriched starter infant formula: A randomized, double-blind, multicentre clinical trial. Clin Nutr. 2019;38:1023-30. doi: 10.1016/j.clnu.2018.05.015.