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The impact of commercial human infant formula on fecal attributes in a weanling pig model

K.A. Barry [a], N.D. Fastinger [a,1], J. Folador [a,2], M.L. Bozych [a], M.J. Kullen [b], G.C. Fahey Jr. [a,*]

[a] *Department of Animal Sciences, University of Illinois at Urbana-Champaign, 1207 W. Gregory Dr., Urbana, IL 61801, USA*
[b] *Wyeth Nutrition, 500 Arcola Road, Collegeville, PA 19426, USA*

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Abstract

An experiment to assess the influence of commercially available infant formulas on characteristics of feces in a weanling piglet model was conducted. Seven commercial infant formulas [whey, whey + α-lactalbumin®, whey + α-lactalbumin + oligofructose, whey + GOS + polyfructose, whey protein concentrate 1 (with 27.3% acid hydrolyzed fat), whey protein concentrate 2 (with 29.4% acid hydrolyzed fat), and enzymatically hydrolyzed whey protein concentrate] were offered to weanling piglets ad libitum for 14 d. Fecal attributes that were assessed include: consistency, color, odor, dry matter, organic matter, pH, biogenic amines, short- and branched-chain fatty acids, phenols, indoles, and ammonia. Overall, there were very few differences among treatment groups in fecal attributes assessed. Minor differences in the concentrations of fecal acetate, valerate, putrescine, and cadaverine were observed in piglets fed GOS + polyfructose-containing formula. However, stool consistency, score, odor, pH, ammonia, phenols, and indoles were similar among treatment groups. Diet had an effect on fecal dry matter percentage, organic matter percentage, color, and 4 week body weights of the piglets. The results obtained in this experiment demonstrate that healthy gut function is prevalent in weanling piglets consuming the experimental treatments.

Keywords: Piglet; Infant formula; Whey; Oligofructose; Fecal characteristics

1. Introduction

Previous work has shown that feeding practices and infant formula components can influence the various attributes of the feces of infants. Fecal characteristics that are impacted by infant feeding include the following: stool consistency and associated defecation frequency, stool color, stool pH, as well as other stool chemical properties. Fecal pH values of human milk-fed infants tend to be lower than those of their formula-fed counterparts (Kleeson et al., 1995; Simhon et al., 1982; Bullen et al., 1977), and recent experiments have demonstrated that non-digestible, fermentable oligosaccharide-supplemented formulas significantly decrease stool pH relative to control formulas. Several studies have demonstrated that formula components influence fecal color in infants; green stools are more commonly observed in response to feeding higher iron ($\geqslant$12 mg/L) formulas (Hyams et al., 1995; Malacaman et al., 1985), while casein-dominant formula results in browner stools when compared to the more yellow stools from infants fed whey-predominant formula.

The passage of hard stools can cause substantial distress to infants and their caregivers (Potts and Sesney, 1992). A variety of studies have demonstrated that hard stools are more frequently associated with formula feeding than with

* Corresponding author. Tel.: +1 217 333 2361; fax: +1 217 333 7861.
E-mail address: gcfahey@uiuc.edu (G.C. Fahey Jr.).
[1] Present address: Procter & Gamble Pet Care, 6571 St. Route 503 N, Lewisburg, OH 45338, USA.
[2] Present address: BASA – Brasilia Alimentos S/A, N. Rural de Tabatinga lote 25, Planaltina 73301-970, DF, Brazil.

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breastfeeding (Hyams et al., 1995; Quinlan et al, 1995; Weaver et al., 1988). Further, infant formula composition also appears to alter fecal consistency and defecation frequency (Duman et al., 2000; Forsyth et al., 1999; Hyams et al., 1995; Malacaman et al., 1985). Compared with control formulas, the feeding of infant formulas containing the following components resulted in a softer stool consistency: polyunsaturated fatty acids (Forsyth et al., 1999), hydrolyzed protein vs. intact protein (Hyams et al., 1995), 50% sn-2 palmitate formula vs. control (high sn-1 and sn-3 palmitate) formula (Kennedy et al., 1999), and whey-dominant protein compared to casein-dominant (Malacaman et al., 1985). Further, several recent experiments have demonstrated that inclusion of non-digestible oligosaccharides in infant formulas increases defecation frequency and results in a softer stool that is more similar to that of human milk-fed infants (Bettler and Euler, 2006; Boehm et al., 2002; Schmelzle et al., 2003; Moro et al., 2002).

As an initial step in understanding and defining the impact of formula feeding on fecal attributes, the purpose of this work was to assess the impact of feeding several different formulas on fecal attributes of weanling pigs,

Table 1
Ingredient inclusion in commercial infant formulas

Ingredient	Diet[a]						
	Whey	Whey + α-Lac[b]	Whey + α-Lac + oligofructose	Whey + GOS + polyfructose[c]	Whey protein concentrate 1	Whey protein concentrate 2	Enzymatically hydrolyzed whey protein concentrate
Carbohydrate							
Lactose	X	X	X	X	X	X	X
Dietary fibers							
GOS				X			
Polyfructose				X			
P-95 Oligofructose			X				
Inositol		X	X		X	X	X
Corn maltodextrin							X
Protein							
Whey powder				X			
Whey protein concentrate					X	X	
Enzymatically hydrolyzed reduced minerals whey							X
Reduced minerals whey	X	X	X				
Taurine	X	X	X	X	X	X	X
L-Arginine				X			
L-Carnitine					X	X	X
α-Lactalbumin®		X	X				
Lipid							
Vegetable oils	X	X	X	X	X		X
Palm	X						
Palm olein					X		X
Soy	X				X	X	X
Coconut	X				X	X	X
High oleic sunflower	X				X		X
High oleic safflower	X					X	X
Vegetable oil mix		X	X				
Egg lipid				X			
Fish oil				X			
M. alpina oil					X	X	X
C. cohnii oil					X	X	X
Long chain polyunsaturates (DHA, ARA)[d]	X	X	X				
Monoglycerides	X	X	X				

[a] Trade names for infant formulas are as follows: Whey = SMA gold, Whey + α-Lac = SMA gold with α-lactalbumin, Whey + α-Lac + oligofructose = SMA gold with α-lactalbumin plus 3.0 g/L oligofructose, Whey + GOS + polyfructose = Milupa Aptimil, Whey protein concentrate 1 = Enfamil Lipil, Whey protein concentrate 2 = Similac Advance, Enzymatically hydrolyzed whey protein concentrate = Nestle Good Start. All diets contain nucleotides, vitamins, and minerals to meet the nutritional requirements of human infants.

[b] α-Lactalbumin®.

[c] GOS = galactooligosaccharides.

[d] DHA = docosahexaenoic acid, ARA = arachidonic acid.

particularly that of an infant formula with the addition of a specific prebiotic oligosaccharide, namely P-95 Raftilose (trade name Beneo), to an infant formula at an inclusion rate of 3 g/L. Weanling pigs were chosen to represent human infants in this study as they are the animal species most similar physiologically and metabolically to humans (Anzenbacherovea et al., 2003).

2. Materials and methods

Experimental design. Fifty-six weanling piglets ((Yorkshire × Landrace) × Duroc) obtained from 9 litters (age 14–17 d, 37 males and 19 females; average initial weight 5.56 ± 0.97 kg) were randomly assigned to one of seven diets by litter. Piglets were individually housed in stainless steel metabolism cages with wire mesh floors covered in one corner by Dri-Dek (Kendall Products, Naples, FL), with drip pans for excreta collection located under the wire mesh. Piglets were kept in an environmentally controlled room (held at 26.7 °C with a 16:8 h light:dark cycle illuminated by fluorescent lighting) with free access to water. All animal care procedures were approved by the Institutional Animal Care and Use Committee, University of Illinois at Urbana-Champaign.

Dietary treatments consisted of seven powdered infant formulas prepared under commercial conditions: whey-dominant powdered diet (European SMA Gold: Wyeth Nutritionals, Askeaton, Ireland – Whey); whey-dominant powdered diet with added α-lactalbumin® (Latin American SMA Gold plus α-lactalbumin: Wyeth Nutritionals, Naucalpan, Mexico – Whey + α-Lac); whey-dominant powdered diet with added α-lactalbumin and 3.0 g/L oligofructose [Latin American SMA Gold plus α-lactalbumin with P-95 Raftilose (now known as Beneo® oligofructose, Orafti, Belgium): Wyeth Nutritionals, Naucalpan, Mexico – Whey + α-Lac + oligofructose]; whey-based powdered diet with galactooligosaccharides (GOS) and polyfructose (Aptimil First: Milupa, Deansgrange, Ireland – Whey + GOS + polyfructose); whey protein concentrate-based powdered diet with 27% acid hydrolyzed fat (Enfamil Lipil: Mead Johnson and Co., Evansville, IN, USA – Whey protein concentrate (1); whey protein concentrate-based powdered diet with 29% acid hydrolyzed fat (Similac Advance: Ross Products Division, Columbus, OH, USA – Whey protein concentrate (2); and enzymatically hydrolyzed whey protein concentrate-based powdered diet (Nestle Good Start: Nestle USA, Glendale, CA, USA – Enzymatically hydrolyzed whey protein concentrate). The ingredients that are included (as listed by the manufacturers) in the seven diets are presented in Table 1.

On study day 0 (day 14–17 of life of the pig), piglets were offered 500 mL milk replacer (ProNurse Specialty Milk Replacer, Purina Mills, St. Louis, MO) upon arrival to provide nourishment and to maintain similarity in fecal attributes to that of piglets consuming sow′s milk before starting the piglets on their assigned dietary treatments. Piglets then were fed their assigned diet for 13 d before sample collection. Treatment diets were offered ad libitum at 0600, 1000, 1400, 1800, and 2200 h daily after reconstitution with water as per the manufacturers′ instructions. All diets were administered to piglets ad libitum in pre-weighed bottles attached to gravity-fill bowls. Feed refusals were weighed back once the piglet was satiated. Feed intake, fecal output, and fecal attributes were measured daily. Body weight was recorded at the beginning (day 0) and end (day 14) of the experimental period. The adaptation period was followed by a 1-d collection period during which all freshly voided feces from each pig were collected for determination of dry matter, organic matter, ammonia, phenol/indole, and biogenic amine concentrations.

Sample collection. Individual samples of freshly voided feces were collected into sterile sampling bags (Whirl-Pac, Pioneer Container Corp., Cedarburg, WI) within 15 min of defecation and processed immediately in order to minimize any loss of volatile components. Fecal samples were weighed before measuring pH, using an AP10 pH meter from Denver Instrument (Fischer Scientific, Inc., Pittsburgh, PA) with a FUTURA refillable combination AgCl electrode with rugged (semi-flat) bulb (Beckman Instruments, Inc., Fullerton, CA). Fecal consistency was scored using a scale of 1–5, with 1 being a watery liquid, 2 being loose or mushy, 3 being soft, 4 being formed, and 5 being dry, formed feces. Fecal color was recorded using a scale of 1–6, with 1 as yellow, 2 as green, 3 as brown, 4 as greenish-black, 5 as brownish-black, and 6 as black. Stool odor was scored using a scale of 1–4, with 1 being no odor, 2 being mild odor, 3 being moderate odor, and 4 being strong odor. Fecal aliquots were sealed in clean centrifuge tubes and stored at −20 °C for subsequent analyses.

Chemical analyses. Diets were analyzed for dry matter (DM), organic matter (OM) (AOAC, 1985), crude protein (CP) (from Leco nitrogen values; AOAC, 1995), acid hydrolyzed fat (Budde, 1952; AACC, 1983), gross energy (GE) (Parr Instrument Co., Moline, IL; Parr Instrument Manuals), amino acids (using a Beckman 6300 amino acid analyzer [Beckman Coulter, Inc., Fullerton, CA] at the University of Missouri Experiment Station Chemical Laboratories using methods outlined by AOAC (1995)), minerals (at the University of Missouri Experiment Station Chemical Laboratories using methods outlined by AOAC (1995)), total phosphorus (AOAC, 1995), free and bound monosaccharides (Smiricky et al., 2002; Bourquin et al., 1990; Hoebler et al., 1989), free oligosaccharides (Smiricky et al., 2002), and long chain fatty acids (Lepage and Roy, 1986). All procedures were performed in duplicate except for amino acids, which were performed singly. To maintain quality control during chemical analyses, the error between duplicate samples was determined, and, if error exceeded 5%, the assay was repeated. Fresh feces were analyzed for DM, OM (AOAC, 1995), pH, phenols, indoles (Flickinger et al., 2003), and biogenic amines (Flickinger et al., 2003).

Statistical analyses. Data were analyzed using a randomized block design. Data for dry matter, organic matter, mineral, crude protein, acid hydrolyzed fat, and carbohydrate intake were analyzed using mixed model procedures and a Tukey adjustment of SAS (SAS, 1985). Study day 14 data for fecal DM, fecal OM, pH, body weight, phenols, indoles, biogenic amines, short-chain fatty acids, and branched-chain fatty acids were analyzed using Mixed model procedures and a Tukey adjustment of SAS, with d 0 data considered as covariates to account for variations within animal. Outlier data were removed from analysis after analyzing data through the Univariate procedure of SAS to produce a normal probability plot based on residual data and visual inspection of the raw data. Outlier data were defined as data points 3 or more standard deviations from the mean of the raw data. Stool consistency, color, and odor were analyzed as categorical data by the GLIMMIX procedure of SAS. The model contained the fixed effect of diet and the random effects of pig, day, and block. Treatment differences for all statistical analyses were assigned using the least significant difference calculated from standard errors using the mixed models procedure. Significant differences were analyzed at $P < 0.05$, and trends were analyzed at $P < 0.10$.

3. Results

A complete listing of ingredients of all test diets as they appeared on the label can be found in Table 1, and analytical values for the test diets appear in Table 2. All diets contained lactose, taurine, a source of docosahexaenoic acid (DHA), and a blend of vegetable oils. All powdered diets had similar DM, OM, CP, and GE concentrations expressed as percentages of the diet. Whey protein concentrate 1 had the highest acid hydrolyzed fat concentration at 29.4% DMB, whereas whey + α-Lac + oligofructose had the lowest acid hydrolyzed fat concentration at 25.2% DMB. Amino acid (AA) values reflect analyzed CP values. The AA concentrations in these diets are formulated to meet human infant requirements, and the amounts present in the formulas are lower than the NRC (1998) recommendations for swine of the same weight.

Linoleic acid values were highest for whey protein concentrate 2 (51.33 mg/g), and lowest for whey + GOS +

Table 2
Compositional analyses of infant formulas

Item	Diet[a]						
	Whey	Whey + α-Lac[b]	Whey + α-Lac + oligofructose	Whey + GOS + polyfructose[c]	Whey protein concentrate 1	Whey protein concentrate 2	Enzymatically hydrolyzed whey protein concentrate
Dry matter,%	98.8	98.5	98.4	99.0	97.9	98.2	98.5
	% dry matter basis						
Organic matter	97.5	97.4	97.5	97.5	96.5	96.8	97.3
Crude protein	11.8	11.1	10.8	10.6	11.0	11.3	11.6
Acid hydrolyzed fat	26.8	26.1	25.2	26.4	27.3	29.4	27.5
	kcal/g						
Gross energy	5.5	5.5	5.5	5.4	5.4	5.6	5.5
	Amino acids[d], g/100 g						
Total AA	11.33	10.83	10.73	10.06	10.24	11.27	11.89
Total EAA	6.11	5.84	5.77	5.37	5.51	6.05	6.50
Total NEAA	5.21	4.99	4.96	4.69	4.73	5.22	5.40
	Fatty acids[e], mg/g						
Linoleic acid	36.49	32.72	32.98	28.12	38.91	51.33	42.93
ARA	0.82	0.74	0.76	0.77	1.66	0.91	1.37
DHA	0.48	0.44	0.45	0.50	0.84	0.39	0.70
EPA	0.00	0.00	0.00	0.11	0.00	0.00	0.00
Total SFA	106.02	102.71	102.78	99.51	102.78	107.56	104.94
Total MUFA	104.22	93.95	94.88	107.30	97.81	112.86	78.92
Total n-3 PUFA	4.08	3.62	3.66	5.11	4.54	5.61	4.99
Total n-6 PUFA	37.50	33.62	33.88	29.50	40.95	52.44	44.58
n-3:n-6 ratio	0.11	0.11	0.11	0.17	0.11	0.11	0.11
Total PUFA	42.66	37.95	38.27	35.18	46.28	59.77	50.32
Total fatty acids	263.70	244.32	245.66	249.80	261.53	269.77	245.69
	Free monosaccharides, μg/g						
Inositol	3635.06	3633.03	3756.46	3403.15	3790.97	3622.68	3499.30
Sorbitol	60.88	36.05	44.46	37.60	45.92	41.52	43.91
Arabinose	9.62	0.00	0.00	12.62	3.06	2.80	5.58
Galactose	2470.70	570.42	538.57	1928.68	3701.15	578.52	5951.83
Glucose	550.53	167.04	201.20	9528.65	1209.06	385.16	2186.57
Fructose	0.00	0.00	1057.04	0.00	0.00	0.00	0.00
Total free monosaccharides	6763.86	4412.63	5603.82	14936.18	8760.62	4640.36	11700.90
	Free oligosaccharides, μg/g						
Lactose	1979.81	2120.63	2016.96	2052.10	2113.94	1900.39	1430.05
GF2 (kestose)	0.00	0.00	0.00	0.00	0.00	0.00	10.59
Total free oligosaccharides	1979.81	2120.63	2016.96	2052.10	2113.94	1900.39	1440.64
	Hydrolyzed monosaccharides, mg/g						
Galactose	328.68	344.44	336.30	324.60	331.71	296.35	218.58
Glucose	322.80	337.57	329.96	320.29	323.11	285.20	411.75
Total hydrolyzed monosaccharides	651.48	682.01	664.26	644.89	654.83	581.54	630.33

[a] Trade names for infant formulas are as follows: Whey = SMA gold, Whey + α-Lac = SMA gold with α-lactalbumin, Whey + α-Lac + oligofructose = SMA gold with α-lactalbumin plus 3.0 g/L oligofructose, Whey + GOS + polyfructose = Milupa Aptimil, Whey protein concentrate 1 = Enfamil Lipil, Whey protein concentrate 2 = Similac Advance, Enzymatically hydrolyzed whey protein concentrate = Nestle Good Start.
[b] α-Lactalbumin®.
[c] GOS = Galactooligosaccharides.
[d] AA = Amino acid; EAA = Essential amino acid; NEAA = Nonessential amino acid.
[e] ARA = Arachidonic acid; DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid; SFA = Saturated fatty acid; MUFA = Monounsaturated fatty acid; n-3 = Omega 3; n-6 = Omega 6; PUFA = Polyunsaturated fatty acid.

polyfructose (28.12 mg/g) (Table 2). Arachidonic acid (ARA, 1.66 mg/g) and DHA (0.84 mg/g) were highest for whey protein concentrate 1. Whey + GOS + polyfructose was the only diet that contained EPA. Total SFA, MUFA, n-3 PUFA, n-6 PUFA, PUFA, and fatty acids were highest in the whey protein concentrate 2 diet.

Free galactose was highest (5951.83 μg/g) in enzymatically hydrolyzed whey protein concentrate, and free glucose was highest (9528.65 μg/g) in whey + GOS + polyfructose. Whey + GOS + polyfructose also had the highest total free monosaccharide value. Lactose and total free oligosaccharides were highest in whey + α-Lac. Enzymatically

Table 3
Average daily intakes of infant formulas

Item	Diet[a]						
	Whey	Whey + α-Lac[b]	Whey + α-Lac + oligofructose	Whey + GOS + polyfructose[c]	Whey protein concentrate 1	Whey protein concentrate 2	Enzymatically hydrolyzed whey protein concentrate
Feed intake, g/d	2525 ± 328	2613 ± 328	2640 ± 328	2243 ± 337	2238 ± 337	2711 ± 332	2179 ± 337
	g/d, dry matter basis						
Dry matter	310 ± 30	319 ± 30	330 ± 30	308 ± 31	272 ± 31	330 ± 30	272 ± 31
Organic matter	302 ± 29	311 ± 29	322 ± 29	300 ± 30	262 ± 30	320 ± 30	265 ± 30
Mineral	8.0 ± 0.8[d]	8.2 ± 0.8[d]	8.4 ± 0.8[d]	7.7 ± 0.8[d]	9.5 ± 0.8[d,e]	10.7 ± 0.8[e]	7.2 ± 0.8[d]
Crude protein	36.6 ± 3.3	35.4 ± 3.3	35.6 ± 3.3	32.6 ± 3.5	30.0 ± 3.5	37.5 ± 3.4	31.5 ± 3.5
Acid hydrolyzed fat	83.0 ± 7.9[f,g]	83.3 ± 7.9[f,g]	83.3 ± 7.9[f,g]	81.2 ± 8.3[f,g]	74.2 ± 8.3[f]	97.0 ± 8.1[g]	74.6 ± 8.3[f]
Carbohydrate	205.7 ± 19.8	221.7 ± 19.8	223.5 ± 19.8	202.3 ± 20.8	181.2 ± 20.8	195.3 ± 20.2	175.4 ± 20.8

[a] Values are means ± standard error. $n = 7$ (whey + GOS + polyfructose, whey protein concentrate 1, whey protein concentrate 2), 8 (enzymatically hydrolyzed whey protein concentrate), and 9 (whey, whey + α-Lac, whey + α-Lac + oligofructose).
[b] α-Lactalbumin®.
[c] GOS = Galactooligosaccharides.
[d,e] Superscripts in the same row denote differences ($P < 0.05$) among treatments.
[f,g] Superscripts in the same row denote a trend ($P < 0.10$) among treatments.

hydrolyzed whey protein concentrate was the only treatment with a detectable kestose value. Hydrolyzed galactose was highest (344.44 mg/g) in whey + α-Lac, and hydrolyzed glucose was highest (411.75 mg/g) in enzymatically hydrolyzed whey protein concentrate. Total hydrolyzed monosaccharides were highest in the whey + α-Lac treatment.

No differences were observed among treatments in DM, OM, CP, or carbohydrate intakes, as noted in Table 3. Mineral consumption was highest for piglets consuming the whey protein concentrate 2 treatment. Also, piglets consuming the whey protein concentrate 2 treatment had higher intakes of fat.

Piglets consuming the enzymatically hydrolyzed whey protein concentrate diet had the lowest body weights ($P < 0.10$) after 14 d of treatment, as noted in Table 4. These piglets also consumed numerically less formula than all other treatment groups.

Fecal OM percentage at day 14 was lowest ($P < 0.05$) for the whey protein concentrate 2 treatment, and highest for the whey + α-Lac + oligofructose and whey + GOS + polyfructose treatments. There were no differences noted after 14 d of treatment in fecal pH. Piglets fed all treatments produced feces with an average score of soft and an average stool odor of mild. Piglets consuming whey + GOS + polyfructose and whey + α-Lac treatments produced green colored stools, whereas all other treatments produced brown stools.

No differences were noted among treatments in concentrations of ammonia, 4-methyl phenol, indole, or 3-methyl indole, as noted in Table 5. In addition, fecal samples were

Table 4
Body weight, fecal composition, and fecal characteristics of weanling piglets consuming infant formulas

Item	Diet[a]						
	Whey	Whey + α-Lac[b]	Whey + α-Lac + oligofructose	Whey + GOS + polyfructose[c]	Whey protein concentrate 1	Whey protein concentrate 2	Enzymatically hydrolyzed whey protein concentrate
Body weight at day 14, kg	8.4 ± 0.3[d,e]	8.3 ± 0.3[d,e]	8.3 ± 0.3[d,e]	8.1 ± 0.3[d,e]	8.0 ± 0.3[d,e]	8.7 ± 0.3[e]	7.5 ± 0.3[d]
Fecal dry matter at day 14,%	35.5 ± 2.8[g,h]	41.4 ± 2.8[h]	38.2 ± 3.1[g,h]	27.9 ± 3.2[g]	35.9 ± 3.2[g,h]	38.9 ± 3.0[g,h]	35.7 ± 3.4[g,h]
Fecal organic matter at day 14,%	89.1 ± 1.4[e,f]	88.3 ± 1.4[e,f]	90.7 ± 1.5[f]	92.5 ± 1.7[f]	84.4 ± 1.6[d,e]	82.5 ± 1.5[d]	88.2 ± 1.6[e,f]
Fecal pH at day 14	6.85 ± 0.12	6.85 ± 0.12	6.55 ± 0.13	6.72 ± 0.14	6.78 ± 0.14	6.90 ± 0.13	6.94 ± 0.15
Stool consistency	3.6 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	3.2 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	3.6 ± 0.2
Stool color	2.6 ± 0.2[d,e]	2.5 ± 0.2[d]	2.7 ± 0.2[d,e]	2.4 ± 0.2[d]	3.1 ± 0.2[e]	3.3 ± 0.2[e]	3.3 ± 0.2[e]
Stool odor	2.3 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.3 ± 0.1

[a] Values are means ± standard error. $n = 7$ (whey + GOS + polyfructose, whey protein concentrate 1, whey protein concentrate 2), 8 (enzymatically hydrolyzed whey protein concentrate), and 9 (whey, whey + α-Lac, whey + α-Lac + oligofructose).
[b] α-Lactalbumin®.
[c] GOS = Galactooligosaccharides.
[d,e,f] Superscripts in the same row denote differences ($P < 0.05$) among treatments.
[g,h] Superscripts in the same row denote a trend ($P < 0.10$) among treatments.

Table 5
Concentrations of ammonia, phenols, and indoles on day 14 in feces of weanling piglets consuming infant formulas

Item	Diet[a,b]						
	Whey	Whey + α-Lac[c]	Whey + α-Lac + oligofructose	Whey + GOS + polyfructose[d]	Whey protein concentrate 1	Whey protein concentrate 2	Enzymatically hydrolyzed whey protein concentrate
	mg/g						
Ammonia	3.3 ± 0.4	3.1 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	3.8 ± 0.4	4.0 ± 0.4	4.2 ± 0.5
	μg/g						
4-methyl phenol	204.5 ± 50.2	256.3 ± 51.7	270.0 ± 46.8	206.8 ± 50.4	277.5 ± 50.2	290.5 ± 44.3	261.4 ± 54.1
Indole	64.1 ± 28.3	19.0 ± 28.8	56.3 ± 25.3	52.4 ± 25.4	84.8 ± 27.8	94.4 ± 23.7	57.5 ± 30.0
3-methyl indole	258.7 ± 68.0	244.1 ± 68.8	225.6 ± 66.8	134.19 ± 68.8	155.8 ± 68.4	209.7 ± 65.5	216.0 ± 70.9

[a] Values are means ± standard error. $n = 7$ (whey + GOS + polyfructose, whey protein concentrate 1, whey protein concentrate 2), 8 (enzymatically hydrolyzed whey protein concentrate), and 9 (whey, whey + α-Lac, whey + α-Lac + oligofructose).
[b] Standard errors that result in a negative mean value, while biologically bounded by zero, can result from adjustments made in their calculation.
[c] α-Lactalbumin®.
[d] GOS = Galactooligosaccharides.

Table 6
Biogenic amine concentrations on day 14 in feces of weanling piglets consuming infant formulas

Item	Diet[a,b]						
	Whey	Whey + α-Lac[c]	Whey + α-Lac + oligofructose	Whey + GOS + polyfructose[d]	Whey protein concentrate 1	Whey protein concentrate 2	Enzymatically hydrolyzed whey protein concentrate
	μmol/g						
Agmatine	0.06 ± 0.50	1.21 ± 0.59	1.00 ± 0.59	0.06 ± 0.55	0.80 ± 0.67	0.83 ± 0.60	−0.01 ± 0.66
Tryptamine	0.06 ± 0.29	0.31 ± 0.34	0.72 ± 0.32	0.83 ± 0.27	0.62 ± 0.37	0.29 ± 0.30	1.09 ± 0.37
Phenylethylamine	0.23 ± 0.11	0.05 ± 0.13	0.00 ± 0.13	0.01 ± 0.11	0.04 ± 0.15	0.11 ± 0.12	0.11 ± 0.14
Putrescine	0.21 ± 3.10[f]	−0.01 ± 3.59[e,f]	1.79 ± 3.40[f]	16.86 ± 2.90[g]	0.59 ± 3.76[f]	2.25 ± 3.13[f]	0.68 ± 3.74[f]
Cadaverine	26.39 ± 17.28[f]	7.19 ± 20.44[f]	41.16 ± 20.90[f]	180.86 ± 17.34[g]	18.31 ± 23.19[f]	28.48 ± 18.78[f]	80.37 ± 23.11[f]
Histamine	0.32 ± 0.35	0.00 ± 0.43	0.24 ± 0.43	0.13 ± 0.33	0.02 ± 0.45	1.05 ± 0.35	0.48 ± 0.50
Tyramine	0.80 ± 0.64	0.72 ± 0.74	1.42 ± 0.67	1.33 ± 0.59	0.26 ± 0.78	1.06 ± 0.63	0.80 ± 0.78
Spermidine	0.74 ± 0.54	0.98 ± 0.58	1.16 ± 0.54	1.20 ± 0.54	1.29 ± 0.61	1.11 ± 0.52	1.90 ± 0.61
Spermine	0.56 ± 0.42	1.04 ± 0.56	0.65 ± 0.42	0.65 ± 0.50	0.50 ± 0.52	1.06 ± 0.41	1.43 ± 0.50

[a] Values are means ± standard error. $n = 7$ (whey + GOS + polyfructose, whey protein concentrate 1, whey protein concentrate 2), 8 (enzymatically hydrolyzed whey protein concentrate), and 9 (whey, whey + α-Lac, whey + α-Lac + oligofructose).
[b] Standard errors that result in a negative mean value, while biologically bounded by zero, can result from adjustments made in their calculation.
[c] α-Lactalbumin®.
[d] GOS = Galactooligosaccharides.
[e] Putrescine has a negative concentration due to the use of day 1 concentrations as a covariate for the final concentrations presented in this table. Putrescine concentrations in fecal samples from piglets consuming the Whey + α-Lac treatment fell below the day 1 concentrations. While this value is biologically impossible, it is important to note that the value is very similar to zero and that fecal putrescine concentrations are greatly impacted by this treatment.
[f,g] Superscripts in the same row denote differences ($P < 0.05$) among treatments.

analyzed for phenol and 7-methyl indole. These compounds were not detectable using known standards as a comparison and, thus, do not appear in the data table.

No differences were noted among treatments in concentrations of agmatine, tryptamine, phenylethylamine, histamine, tyramine, spermidine, or spermine, as noted in Table 6. Piglets consuming the whey + GOS + polyfructose treatment produced significantly more putrescine (16.86 μmol/g) and cadaverine (180.86 μmol/g) after 14 d of treatment than did piglets in any other treatment group. At day 14 of treatment, all other groups had very low concentrations of putrescine (−0.01 to 2.25 μmol/g) and, compared with the whey + GOS + polyfructose treatment (180.86 μmol/g), cadaverine concentrations were lower (7.19 to 80.37 μmol/g, $P < 0.05$) in all other treatment groups.

Acetate concentrations were highest ($P < 0.05$) for the whey + GOS + polyfructose treatment, as noted in Table 7. No differences were noted among treatments in concentrations of propionate, butyrate, isobutyrate, or isovalerate. At day 14 of treatment, piglets consuming the whey + GOS + polyfructose diet had the highest concentration of valerate in feces.

4. Discussion

Infant formulas are a complex mixture of macro- and micronutrients, partially reflecting the complex mixture of nutrients found in human milk. These formulations are increasing in complexity as research identifies new analytes in breast milk. As can be seen by the number of ingredients

Table 7
Short- and branched-chain fatty acid concentrations on day 14 in feces of weanling piglets consuming infant formulas

Item	Diet[a]						
	Whey	Whey + α-Lac[b]	Whey + α-Lac + oligofructose	Whey + GOS + polyfructose[c]	Whey protein concentrate 1	Whey protein concentrate 2	Enzymatically hydrolyzed whey protein concentrate
	mmol/g						
Acetate	94.5 ± 14.4[d]	78.7 ± 14.4[d]	112.4 ± 15.5[d,e]	181.4 ± 17.6[e]	117.3 ± 16.4[d,e]	123.0 ± 15.4[d,e]	107.2 ± 17.7[d,e]
Propionate	36.5 ± 8.1	19.7 ± 7.3	38.2 ± 10.0	52.8 ± 9.6	36.0 ± 8.9	32.3 ± 12.4	38.6 ± 9.6
Butyrate	21.9 ± 4.0	18.0 ± 4.2	21.5 ± 4.4	25.5 ± 5.9	24.0 ± 4.6	32.4 ± 6.0	22.6 ± 5.4
Isobutyrate	7.0 ± 0.9	5.8 ± 0.9	7.7 ± 1.0	7.9 ± 1.0	7.7 ± 1.0	9.3 ± 1.0	8.9 ± 1.1
Isovalerate	12.9 ± 1.5	10.9 ± 1.6	13.4 ± 1.7	13.2 ± 1.8	13.4 ± 1.8	15.9 ± 1.7	15.4 ± 1.9
Valerate	6.1 ± 1.8[d]	4.9 ± 1.8[d]	11.3 ± 2.0[d,e]	16.3 ± 2.1[e]	7.4 ± 2.1[d]	9.6 ± 2.0[d,e]	10.0 ± 2.2[d,e]

[a] Values are means ± standard error. $n = 7$ (whey + GOS + polyfructose, whey protein concentrate 1, whey protein concentrate 2), 8 (enzymatically hydrolyzed whey protein concentrate), and 9 (whey, whey + α-Lac, whey + α-Lac + oligofructose).
[b] α-Lactalbumin®.
[c] GOS = Galactooligosaccharides.
[d,e] Superscripts in the same row denote differences ($P < 0.05$) among treatments.

listed in Table 1, many different compounds within the same line of products are utilized to achieve a nutritional profile that will properly nourish infants as well as create a stool that has the consistency and characteristics deemed normal for human infants.

Average daily gain of piglets in this study was lower than typical for piglets of the same age fed a diet appropriate for optimal growth. This is related primarily to the protein and AA composition of these formulas relative to what is optimal for rapidly growing pigs. However, all piglets gained weight on these diets, and the rapid growth that is expected of weanling piglets is not considered beneficial in human infants. In a study conducted by Dewey et al. (1992), breastfed infants gained approximately 4007 g (male and female infants combined) over the six-month period from birth to 6 month of age, which calculates to an average daily gain of approximately 22 g/d. In the same study, formula-fed infants gained approximately 4421 g/d (male and female infants combined) over the same time period, which calculates to an average daily gain of approximately 25 g/d. While it appears that formula-fed infants gain more weight over a six-month period, this difference is not significant and may have no biological repercussions. When average daily gains of piglets in this study were calculated, they ranged from 141 to 223 g/d, notably higher than previously published values for human infants. This can be attributed to a higher intake by weanling piglets than would be observed in a human infant.

A soft or mushy stool, as was produced by all treatment groups, is viewed as ideal by parents and researchers alike because these fecal attributes lead to less constipation in infants (Knol et al., 2005). Knol et al. (2005) observed mushy/soft stool consistencies when a whey + GOS + polyfructose diet was administered to human infants, which can be attributed to the addition of GOS and polyfructose to the diets. Whey protein concentrate 1 produced mushy feces in a study conducted with human infants by Hyams et al. (1995), and whey protein concentrate 2 produced soft feces when administered to human infants for 4 weeks (Malacaman et al., 1985). Fecal colors observed in this study were expected to be brown, as all diets were whey-based formulas; however, whey + GOS + polyfructose and whey + α-Lac produced green stools, which are commonly associated with higher concentrations of iron in the diet (Malacaman et al., 1985). All diets were analyzed for and contain iron with a range of 44–98 ppm (dry matter basis). In addition, copper was analyzed in the diets at 1.6–4.5 ppm (dry matter basis). Given that iron is found at a higher concentration than copper, it is likely that iron is the cause of the greener stools.

Fecal pH values observed in this study were higher than previously published values from studies conducted with the same diets in human infants. The pH values were 6.3 for a whey + GOS + polyfructose treatment after a 6 week trial period when administered to human infants (Knol et al., 2005) and 6.18 for a whey treatment after a 14 d trial period (Balmer et al., 1989). The values in the present study are similar to pH values published by Howard et al. (1995) who investigated the addition of 3 g/L FOS to a liquid neonatal piglet diet. When FOS was included, cecal pH was 6.74, while pH was 6.76 without the addition of FOS.

Ammonia production is of major concern in both growing piglets and human infants as it is an odor component and can alter DNA synthesis in the epithelium as it is toxic to cells (Cummings and Macfarlane, 1991). Concentrations of ammonia produced by the weanling piglets in this study at 28 d of age (day 14 of treatment) consuming infant formulas were lower than those of piglets 38 d of age consuming a cornstarch/fishmeal-based diet with or without added inulin (Awati et al., 2006). These results are not surprising, as typical diets fed to weanling piglets are higher in AA due to the higher requirements of AA for piglet growth. The concentration of AA required for human infants is considerably less than that of a weanling piglet and, as such, the AA available for ammonia production would be significantly less. Also, the AA found in these infant formulas may be more digestible than the AA found in the cornstarch/fishmeal

diet, which would decrease ammonia production in piglets consuming infant formula.

Phenolic and indolic compounds are known odor components and are potential carcinogens within the colon when combined with putrefactants (Cummings and Macfarlane, 1991). In the current study, all treatments produced statistically similar concentrations of 4-methyl phenol, indole, and 3-methyl indole, respectively. Phenol and indole data collected in this study correlate well with data published for 30 kg pigs (Rideout et al., 2004). Concentrations at day 14 for *p*-cresol (4-methyl indole), indole, and skatole (3-methyl indole) are very similar for piglets consuming infant diets as well as larger pigs consuming a corn–soybean diet.

Biogenic amines, like phenolic and indolic compounds, are odor components of feces created from the decarboxylation of amino acids in the intestine (Cummings and Macfarlane, 1991). These compounds also are important to the apoptotic signal pathways of normally functioning intestinal cells (Loser et al., 1999; Seiler and Raul, 2005). Concentrations of the biogenic amines tyramine, spermidine, and spermine correlate well with data published on 70 d old piglets fed a corn-based diet (Piva et al., 2002). When comparing putrescine values, concentrations in the present study are low, with the exception of those associated with the whey + GOS + polyfructose treatment. Initial (baseline) fecal samples to allow each animal to serve as its own control (Littell et al., 2006), as fecal metabolite concentrations were expected to change in response to infant formula consumption. In the case of the Whey + α-Lac treatment, concentrations of putrescine in treatment fecal samples dropped below concentrations of putrescine in baseline fecal samples. When the treatment mean was calculated, a negative concentration very similar to zero occurred. While it is biologically impossible to produce negative concentrations of any given substance, it is important to report this value as negative instead of zero as this treatment caused such a dramatic decrease in fecal putrescine concentrations. Cadaverine and histamine concentrations are higher in this study, potentially due to higher lysine and histamine concentrations compared to other AA in infant formulas. When compared to values in adult dogs consuming a corn-based diet containing oligofructose, putrescine values in weanling piglets are low for all treatments except whey + GOS + polyfructose, which contained over twice the amount of putrescine observed in dogs (Flickinger et al., 2003).

Short-chain fatty acids (SCFA) serve to decrease intestinal pH. Butyrate serves as an energy source for epithelial cells in the large intestine (Macfarlane and Cummings, 1991). Branched-chain fatty acids (BCFA) are products of protein fermentation by microflora in the intestine, and serve as a marker for protein degradation in humans (Macfarlane and Cummings, 1991). Due to certain limitations inherent in this study (the high digestibility of infant formula and small amount of fecal material produced as a result, as well as the short study period), a microflora profile was not conducted. However, certain assumptions about the effects of microflora can be made without this profile. The whey + α-Lac treatment consistently produced the lowest concentration of both SCFA and BCFA among all treatments. This may be due to the addition of rapidly absorbed carbohydrates to the diet which would not reach the large intestine in great quantity to be fermented to SCFA or BCFA. The whey + GOS + polyfructose treatment produced the numerically highest propionate concentration and statistically highest acetate and valerate concentrations, likely due to the addition of non-digestible carbohydrates to the diet. The whey protein concentrate 2 treatment produced the numerically highest butyrate, isobutyrate, and isovalerate concentrations. This could be due to a higher crude protein intake, which would allow for more protein to escape absorption in the small intestine to be fermented by the microflora in the large intestine. Fecal SCFA and BCFA concentrations in piglets consuming infant formulas were higher compared to cecal concentrations in piglets approximately 2 weeks younger consuming a liquid diet with or without FOS (Howard et al., 1995). It would appear that both increased processing of the whey source and the inclusion of prebiotic oligosaccharides increase SCFA and BCFA concentrations in piglets.

In conclusion, very few differences were observed among the formula treatment groups in the fecal attributes assessed in this work. Fecal consistency after 14 d of feeding was similar among treatment groups with piglets from all treatments producing feces with an average score of soft. Similarly, the average fecal odor for all groups was determined as "mild." Some minor differences in stool attributes were observed in this work. Concentrations of fecal acetate, valerate, putrescine, and cadaverine were significantly higher in piglets fed the whey + GOS + polyfructose formula. Treatment also had an effect on fecal dry matter percentage, fecal organic matter percentage, fecal color, and 4 week body weights of the piglets, although no consistent trends were observed in these differences. Stool consistency, fecal pH, stool odor, ammonia, phenols, and indoles were similar among treatment groups. Fecal attributes observed in this work were consistent with normal, healthy gut function. These results suggest that the use of an infant formula with the addition of P-95 Raftilose would produce fecal consistencies and attributes similar to formulas currently available to consumers, and that the use of any of the formulas observed in this study would result in normal, healthy gut function in human infants.

Conflict of interest statement

There are no known conflicts of interest at the time of this submission.

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