

## AN EVALUATION OF THE GROWTH OF TERM INFANTS FED FORMULA SUPPLEMENTED WITH FRUCTO-OLIGOSACCHARIDE

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[Received September 1, 2005; Accepted November 21, 2005]

**ABSTRACT:** *The objectives of this work were to evaluate growth and tolerance in infants fed formula supplemented with fructo-oligosaccharide (FOS). Methods: The design was a randomized, masked, multicenter trial. Healthy term infants were randomly assigned to 1 of 3 formulas (a bovine milk-based control formula or identical experimental formulas supplemented with either 1.5 g/L or 3.0 g/L FOS) ad lib for 12 weeks. Anthropometric measurements were recorded at baseline and at 4, 8, and 12 weeks. Adverse events (AEs) and tolerance were recorded throughout the study, and blood samples were drawn at baseline and at 12 weeks for a clinical chemistry panel. Results: The study enrolled 297 infants, of whom 212 completed the trial. On the basis of percentiles and z scores, the infants were found to have grown appropriately, compared with the Centers for Disease Control and Prevention reference ranges. All 3 formulas were judged to be safe and well tolerated based on growth, laboratory data, and AE profiles. It is of clinical significance that the 3.0 g/L FOS group had less constipation ( $P = 0.0333$ ) than the other study groups. Conclusions: Bovine milk-based term formula supplemented with either 1.5 g/L or 3.0 g/L FOS is safe and supports normal growth.*

**KEYWORDS:** Infant formula, Fecal flora, Fructo-oligosaccharide, Prebiotic

**Abbreviations Used:** AE, Adverse Event; ANCOVA, Analysis of Covariance; ANOVA, Analysis of Variance; CDC, Centers for Disease Control and Prevention; FOS, Fructo-oligosaccharides; GOS, Galacto-oligosaccharide; GI, Gastrointestinal; ITT, Intent-to-Treat; NCHS, National Center for Health Statistics

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### INTRODUCTION

The type of feeding an infant receives is thought to influence the gastrointestinal (GI) microflora of infants. Some studies have

demonstrated that human milk-fed infants have more bifidobacteria and lactobacilli and fewer facultative anaerobes, bacteroides, and clostridia than formula-fed infants (Stark and Lee, 1982; Lundequist et al., 1985; Harmsen et al., 2000). Establishing a flora similar to that found in human milk-fed infants is thought to be beneficial, because the predominance of bifidobacteria may provide protection against GI infections and atopic disease (Teitelbaum and Walker, 2002; Mountzouris et al., 2002).

Fructo-oligosaccharides (FOS) are nondigestible carbohydrates that have the ability to influence the composition of the colonic microflora. In vitro studies have demonstrated that FOS is a preferential energy source for bifidobacteria (Wang and Gibson, 1994), and as a result is associated with both an increase in the population of these bacteria and a simultaneous decline in the counts of potentially pathogenic bacteria such as *Escherichia coli* and *Clostridium difficile* (Gibson and Wang 1994). FOS's ability to stimulate bifidobacteria and change the overall composition of the microflora is well documented in adults (Hidaka et al., 1987; Buddington et al., 1996; Kleeson et al., 1997; Gibson et al., 1995). Because of their potentially positive impact on the GI flora, the functional properties of bovine milk-based infant formulas may be improved by the addition of FOS.

Prior to initiating this trial, we performed a search of the medical literature to determine if a growth trial, in which a cow's milk base formula supplemented with FOS, had been published. We found that no such report had been published. We therefore undertook the current study to document safety in an infant population. Growth and tolerance parameters were evaluated over a 12-week period in term infants fed a bovine milk-based formula supplemented with FOS at a concentration of either 1.5 g or 3.0 g per liter. An unsupplemented, but otherwise identical, bovine milk-based formula was used as a control. The study tested the hypothesis that growth, acceptance, and tolerance in infants fed formula supplemented with FOS are similar to these parameters in infants fed unsupplemented formula.

## MATERIALS AND METHODS

This prospective, randomized, masked, multicenter trial was conducted at 17 outpatient physician offices in the United States between December 1999 and April 2001. The objective of the study was to compare growth and safety of a standard bovine milk-based formula with these parameters in 2 identical bovine milk-based formulas supplemented with either 1.5 g/L or 3.0 g/L FOS. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and according to the Guidelines for Good Clinical Practice. The Institutional Review Boards of the participating centers approved the protocol, and written signed informed consent was obtained from the parent(s) or legal guardian.

### Study Population

Healthy term infants  $\leq$  14 days of postnatal age were considered for enrollment if their birth and current weight-for-length were between the 10th and 90th percentiles for age according to growth charts provided by the United States National Center for Health Statistics (NCHS). In addition, the inclusion criteria required the infants to be fed only infant formula at the time of enrollment. Infants were excluded if they had been fed baby food or solid food; had siblings with a history of bovine milk intolerance; had conditions requiring feedings other than those specified in the protocol; had experienced diarrheal episodes; had major congenital malformations; had suspected or documented systemic or congenital infections (such as HIV); had cardiac, respiratory, hematologic, GI, or other systemic disease; or had participated in another trial.

### Study Formula

During the 12 weeks of the trial, the infants received study formula ad lib as their only source of nutrition. The formulas, supplied by Wyeth Nutrition (Collegeville, PA, USA) were in a powdered form that when reconstituted, provided 672 kcal/L (2804 kJ/L). The study formulas were whey dominant bovine milk-based, containing docosahexaenoic and arachidonic acids, and were identical except for the addition of either 1.5 g/L or 3.0 g/L FOS to the experimental formulas. The source of FOS was Raftilose®P95 (manufactured by ORAFTI, Tienen, Belgium), a powder containing  $\geq$  93.2% oligofructose and  $<$ 6.8% glucose + fructose + sucrose. The powder is produced by partial enzymatic hydrolysis of chicory inulin.

Standard formula preparation and storage guidelines were described in the study protocol. The study coordinators at each study site provided education to the infants' caregivers on formula preparation and storage. While consuming the study formulas, infants were permitted to have routine immunizations, prescriptions, over-the-counter medications, and vitamin/mineral supplements.

Infants were randomized to receive one of the 3 study formulas according to a schedule generated by a computerized

randomization/enrollment system. If an infant was withdrawn from the study, the randomization number and corresponding formula were not reassigned to another infant.

### Measurements for Determining Growth and Safety

The primary means for assessing growth were the anthropometric measurements of weight (g), length (cm), and occipitofrontal head circumference (cm), which were determined at baseline and at 4, 8, and 12 weeks. The infants were weighed without a diaper on electronic gram scales, and measurements were recorded to the nearest gram. The scales were balanced and calibrated before the trial and at intervals for the duration of the study. Measurements of length, rounded to the nearest 0.1 cm, were obtained on recumbent length boards and head circumference was measured to the nearest 0.1 cm with a flexible steel measuring tape. Assessments were made at baseline and within  $\pm$  3 days of other time points. These assessment methods have been previously validated (Chierici and Vigi, 1994; Peerson et al., 1993).

The primary parameters for safety assessment were adverse events (AEs) and the infants' acceptance and tolerance of the study formulas. AEs and data for acceptance and tolerance were recorded during visits at 4, 8, and 12 weeks and by telephone contacts at 2, 6, and 10 weeks. An AE was defined as any untoward, undesired, unplanned clinical event, in the form of signs (including findings from the laboratory or from physical examination), symptoms, or disease, occurring in an infant participating in a clinical study, regardless of causal relationship. The assessment of the severity of the AEs and whether the AEs were considered to be related to the study formulas were determined by the investigators at each site. AEs were recorded based on findings from the investigators during the physical examinations and clinical evaluations of the infant and on reports provided in the telephone follow-up calls. In clinical visits and follow-up calls, the parent/guardian was asked the following question, "Since your last phone call or visit, has your baby been ill or experienced an adverse event?" In addition the parent/guardian was asked to describe the infant's overall acceptance and tolerance of the formula as satisfactory or unsatisfactory.

The secondary parameter for safety assessment was a chemistry panel (albumin, blood urea nitrogen, calcium, magnesium, phosphorus, creatinine, triglycerides, low-density lipoprotein, and cholesterol) that was obtained at baseline and at 12 weeks.

### Statistical Analysis

Statistical analyses were performed on a modified intent-to-treat (ITT) basis. The ITT population comprised all the infants who entered the study and received at least one feeding of the study formula. No attempt was made to substitute values for missing data. Any infant with missing values for a variable was excluded from the analysis. The level of significance was set at 0.05 for all tests of main effects and interactions, and the power was set at 90%. Continuous variables were summarized using  $n$  (the ITT), mean, standard deviation, and a 95% confidence

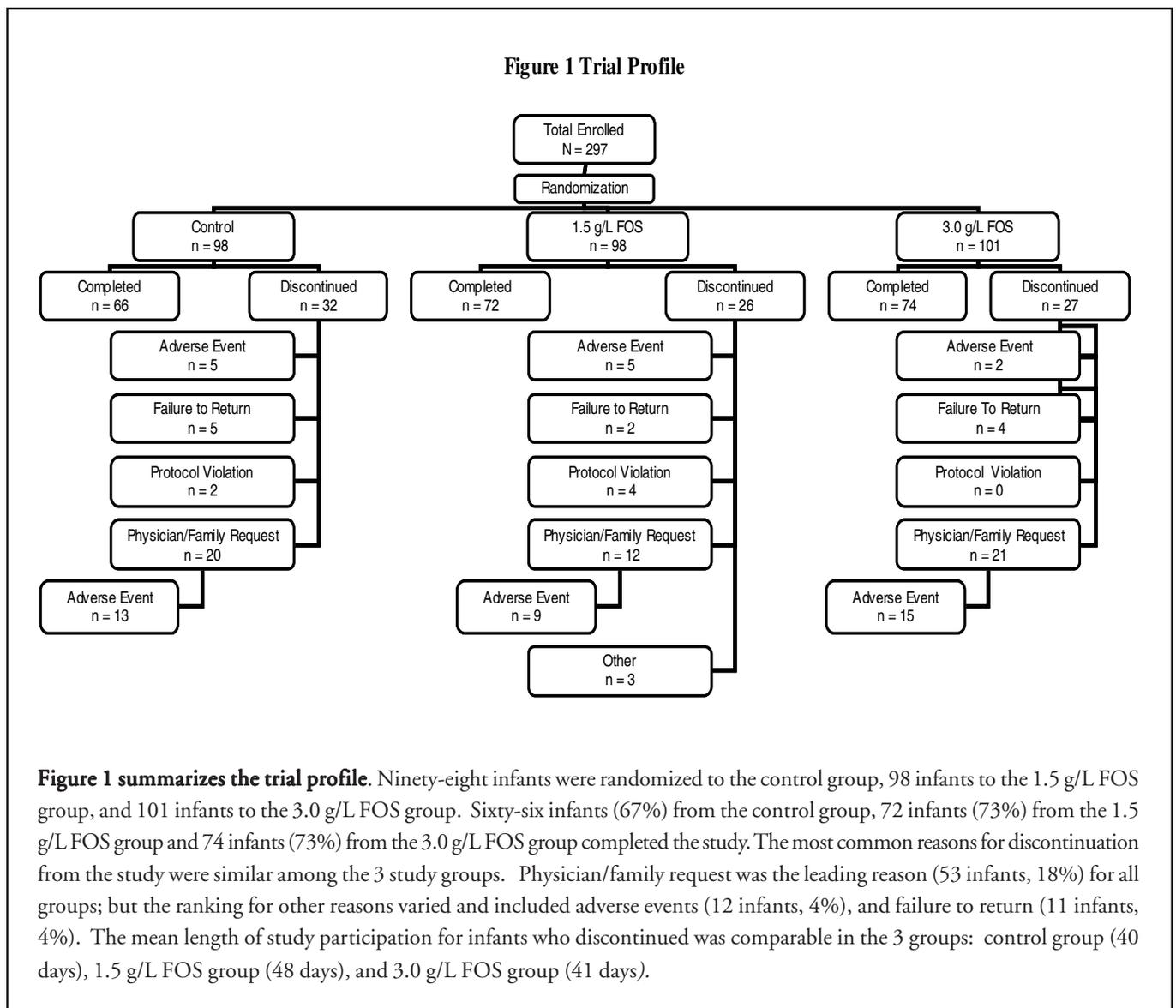
interval around them. Categorical variables were summarized with frequencies and percentages, and continuous variables were summarized by using n, mean, standard deviation, and a 95% confidence interval around the mean.

All data were analyzed using SAS® Statistical Software. Anthropometric data at baseline and at 4, 8, and 12 weeks, and laboratory variables at baseline and at 12 weeks were analyzed using Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA). The size of study infants relative to the United States Centers for Disease Control and Prevention (CDC) reference ranges (Dibley et al., 1989; Kuczmarski et al., 2000) was also assessed for each of the study groups by using z scores for weight, length, and head circumference at baseline, and at 4, 8, and 12 weeks. Z scores were calculated using the Epi-info 2000 software, version 1.1.

Ten AEs were selected to further analyze tolerance. These ten AEs were selected because they are commonly seen in infancy and include the following: abdominal pain, allergic reaction, constipation, diarrhea, flatulence, irritability, loose stools, rash, spitting up, and vomiting. The Fisher exact test was performed on these data and on acceptance and tolerance data to assess differences among the formula groups.

**RESULTS**

The study enrolled 297 infants from 17 centers in the United States. The number of infants enrolled per site ranged from 1 to 77, with an average number of 18 infants per site. Because of a high enrollment at one site, additional analyses were conducted to examine whether the heavy enrollment at this site influenced the overall study findings.



**Table 1. Demographic and Birth Characteristics of Infants Enrolled in the Study**

Variable	Control: n = 98	1.5 g/L FOS: n = 98	3.0 g/L FOS: n = 101
<b>Gender, n (%)</b>			
Male	54 (55)	46 (47)	52 (51)
Female	44 (45)	52 (53)	49 (49)
<b>Race, n (%)</b>			
White	76 (78)	76 (78)	77 (76)
Black	16 (16)	18 (18)	13 (13)
Native American	0 (0)	1 (1)	0 (0)
Asian 0 (0)	0 (0)		1 (1)
Hispanic	6 (6)	2 (2)	7 (7)
Other	0 (0)	1 (1)	3 (3)
<b>Birth Characteristics, mean (SD)</b>			
Weight, g	3428.2 (457.1)	3416.9 (456.9)	3377.2 (477.3)
Length, cm	50.5 (2.7)	50.0 (2.9)	50.3 (2.8)
Head circumference, cm	34.6 (1.6)	34.3 (1.7)	34.2 (1.5)
Gestational age, wk	39.1 (1.1)	39.0 (1.1)	39.0 (1.1)

**Table 2. Anthropometrics at Baseline and After 4, 8, and 12 Weeks of Study Formula Feeding**

	Control	1.5 g/L FOS	3.0 g/L FOS
<b>Weight, g</b>			
<b>Baseline</b>	3486.5 (511.4)	3482.3 (476.6)	3404.9 (437.4)
<b>4 Weeks</b>	4510.9 (615.9)	4556.4 (596.7)	4454.5 (569.5)
<b>8 Weeks</b>	5421.8 (720.9)	5436.7 (686.0)	5367.8 (661.2)
<b>12 Weeks</b>	6115.5 (833.1)	6184.6 (837.2)	6101.5 (736.0)
<b>Length, cm</b>			
<b>Baseline</b>	50.7 (2.2)	50.7 (2.3)	50.5 (2.2)
<b>4 Weeks</b>	54.7 (2.3)	54.6 (2.3)	54.3 (2.3)
<b>*8 Weeks</b>	58.3 (2.6)	57.9 (2.1)	57.5 (2.1)
<b>12 Weeks</b>	61.0 (2.7)	60.4 (2.1)	60.5 (2.4)
<b>Head Circumference, cm</b>			
<b>CF Baseline</b>	35.4 (1.4)	35.5 (1.4)	35.2 (1.3)
<b>4 Weeks</b>	37.7 (1.3)	37.9 (1.3)	37.7 (1.2)
<b>8 Weeks</b>	39.4 (1.3)	39.5 (1.2)	39.4 (1.3)
<b>12 Weeks</b>	40.7 (1.4)	40.8 (1.3)	40.7 (1.3)

Data are given as means (SD).

\*At 8 weeks, the infants in the control group were longer than infants in the 3.0g/L FOS group ( $P = 0.046$ ).

Demographic and birth characteristics of the infants in the study are presented in Table 1. The 3 groups were generally well matched at baseline. Infants were predominantly white 229 (77%), although there were 47 (16%) black infants, 15 (5%) Hispanic infants, and 6 (2%) of other groups, which included Native American and Asian infants. Mean gestational age and anthropometric measurements (birth weight, length, and head circumference) were similar between the 3 groups. The infants in the 3.0 g/L FOS groups were slightly smaller, on average, than the infants in the other two groups, but these differences were not statistically significant.

The anthropometric data collected over the study's 12-week duration are shown in Table 2. The 3 study groups had appropriate mean weight, length, and head circumference gains over this period. Over the 12-week period, the mean weight in the 3 groups increased by nearly 2700 g; mean length increased by 10 cm; and mean head circumference by 5 cm. The mean rate of weight change for all groups was 3.9 g/kg/day over the 12-week period.

At 8 weeks, infants in the control group were longer, on average, than infants in the 3.0 g/L FOS group. Although this difference was statistically significant ( $P = 0.0460$ ), all values were within the normal range. At 12 weeks, the differences in the means for length were smaller and not statistically significant.

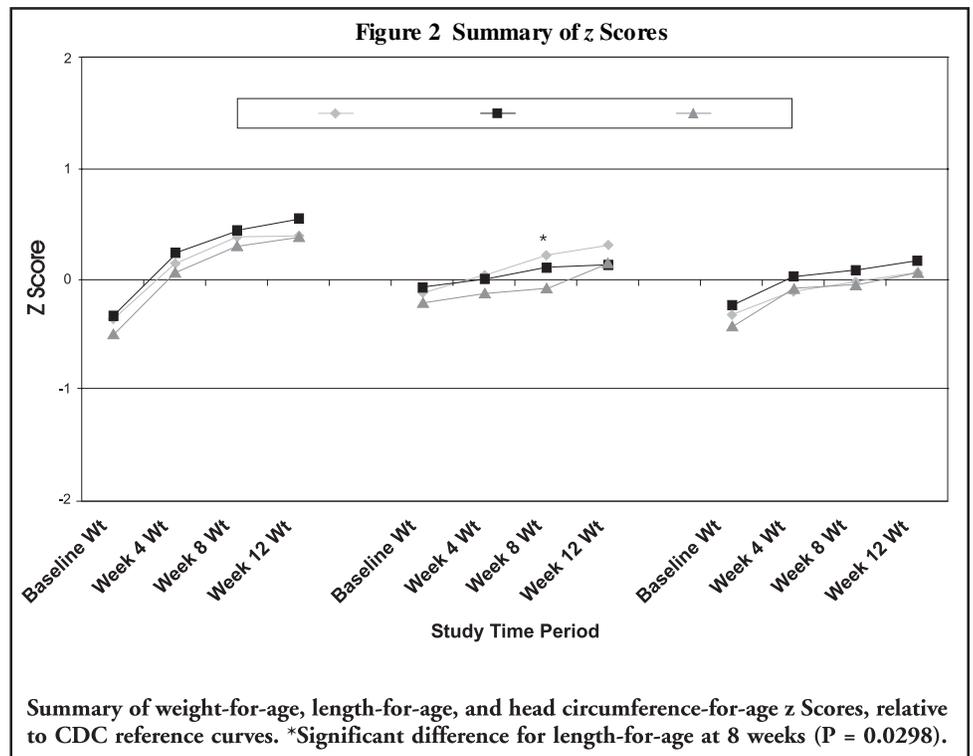
When the site with high enrollment was excluded from the analysis, the anthropometric results showed appropriate growth on all measures across formula groups, and little difference among the groups.

Growth parameters for infants were compared to CDC reference ranges. The study infants, on average, demonstrated adequate growth based on CDC reference ranges for all anthropometric measures over the 12-week study period, and these changes were similar among the formula groups. When plotted on the CDC Growth Charts, the mean weight-for-age percentiles at 12 weeks, increased from 38.0 at baseline to 60.6 in the control group, from 39.3 at baseline to 64.8 in the 1.5 g/L FOS group, and from 34.0 at baseline to 61.2 in the 3.0 g/L FOS group. The differences between the formula groups were not statistically significant at any study time point.

The mean values for length-for-age percentiles at 12 weeks, increased from 46.1 at baseline to 59.3 in the control group, from 47.3 at baseline to 54.8 in the 1.5 g/L FOS group, and from 43.2 at baseline to 54.2 in the 3.0 g/L FOS group. At 8 weeks, the differences in the length-for-age percentiles were statistically significant ( $P = 0.0417$ ), as the length-for-age percentiles for the control group were higher than for the 3.0 g/L FOS formula group. By 12 weeks, however, these differences were not statistically significant.

The mean values for weight-for-length percentiles at 12 weeks increased from 45.3 at baseline to 47.7 for the control group, from 44.7 at baseline to 59.3 for the 1.5 g/L FOS group, and from 43.4 at baseline to 53.3 for the 3.0 g/L FOS group. At 4 weeks, the differences in weight-for-length percentiles were statistically significant ( $P = 0.0332$ ), as the weight-for-length percentiles in the 1.5 g/L FOS group were higher than in the control group. At later time points, however, the differences were not statistically significant.

The mean values for head circumference-for-age percentiles at 12 weeks, increased from 38.5 at baseline to 50.5 for the control group, from 42.0 at baseline to 55.8 for the 1.5 g/L FOS group, and from 35.2 at baseline to 51.7 for the 3.0 g/L FOS group. The differences between the formula groups in head circumference-for-age percentiles were small at all time points and never reached the level of statistical significance.



To supplement the above analyses, individual infant growth variables for infants were compared to CDC reference data by using z scores, and the results are presented in Figure 2. These data reinforce the finding that growth was favorable on all anthropometric measures for each formula group and the magnitude of the changes was very similar among the formula groups. As in the underlying anthropometric data presented earlier, there was a statistically significant difference in length-for-age z scores at Week 8 ( $P = 0.0298$ ), with higher average z scores in the control group than in the 3.0 g/L FOS group, but by Week 12 the differences were again not statistically significant.

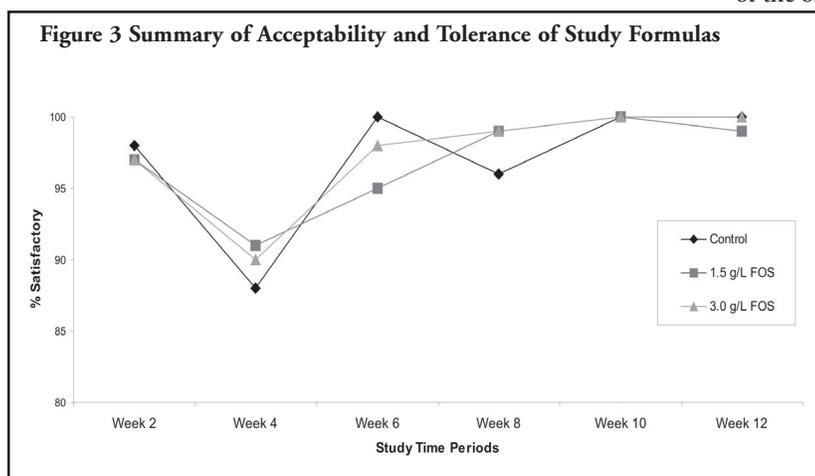
Safety of the formulas was evaluated by comparing the number and type of AEs. The relationship of each event with the study formula was assessed. Fifty-five percent of the infants had at least one formula-related event. The 3.0 g/L FOS group had fewer formula-related AEs than the other formula groups, and the

1.5 g/L FOS group had slightly more AEs than the control group. The majority of the AEs were described as mild and resolved without treatment or sequelae. None of the formula related AEs were considered to be serious. A total of 49 infants were withdrawn from the study in response to adverse events. The number of infants discontinuing the formula due to adverse events was similar across the 3 formula groups.

The statistical significance of differences among formula groups was examined for the 10 pre-selected AEs, mentioned earlier. Constipation was less frequent in the group that received 3.0 g/L FOS ( $P = 0.0333$ ). This comparison was not significant for events of constipation considered by the principal investigator to be formula related. There was also a statistically significant difference among the treatment groups for vomiting ( $P = 0.0162$ ), which was more common in the group that received 1.5 g/L FOS, but this result was not consistent across all analyses and was not statistically significant for events considered to be formula related. While both flatulence and formula-related flatulence incidences in the control group were more than 5% higher than those reported for the 3.0 g/L FOS group, these differences were not statistically significant. There were no significant differences in incidence or diarrhea, loose stools, dehydration or allergic reaction among the three groups.

In order to determine whether the findings were influenced by the high enrollment at one site the analysis was repeated without the data from this site. With the site excluded, the pattern of 10 pre-selected AEs is similar. For constipation, the difference among formula groups remained significant for all events ( $P = 0.0099$ ), with fewer events in the 3.0g/L FOS group. However, in contrast to the previous significant finding with vomiting, the differences among the formula groups were not statistically significant. This suggests the finding of significance reported above may have been influenced by the one site and should be interpreted with caution.

Formula acceptance and tolerance was similar for all 3 groups throughout the study period. No statistically significant differences in formula tolerance and acceptance were observed between the 3 study groups at any time point. Figure 3 describes the results. Laboratory safety assessments showed no significant differences among the 3 formula groups and at baseline and at 12 weeks. For all 3 groups, the mean values were within normal ranges at 12 weeks.



## DISCUSSION

The results of this study support the hypothesis that infants from the three treatment groups would exhibit adequate growth and similar acceptance and tolerance of the study formulas. The growth of the infants consuming FOS-supplemented formulas was compared to a control formula and to the CDC growth charts. The CDC growth charts are a reference population, which includes data from breast-fed and formula-fed infants from a US population. The charts are recommended for use in clinical and research settings to assess size and growth of US infants (Kuczmarski et al., 2003). The infants in our study grew appropriately according to the reference growth charts and comparable to control. The minor differences observed at intermediate time points were not clinically significant. The number of subjects and length of this study meet the American Academy of Pediatrics (1989) guidelines to detect meaningful growth differences.

The acceptance of the study formulas was similar in the 3 groups. The formulas were well tolerated, with the 3.0 g/L FOS group having fewer AEs than the control group. For constipation, the difference among formula groups was significant for all reported events of constipation, with fewer events in the 3.0 g/L FOS group, but this comparison was not significant for constipation events that were considered by the investigator to be formula-related.

Since the initiation of this study, we have identified four randomized clinical trials describing infant formula supplemented with oligosaccharides (Boehm et al., 2002; Moro et al., 2002; Schmelzle et al., 2003; Euler et al. 2005). Three of the studies fed formula supplemented with a mixture of 90% galacto-oligosaccharide (GOS) and 10% FOS. Boehm et al. (2002) studied preterm infants fed 1 g/L of GOS/FOS compared to a control group and a human-milk fed reference group for 28 days. The fecal bifidobacteria counts in preterm infants fed the oligosaccharide mixture and the stool characteristics were similar to those of the human-milk fed reference group. Moro et al., (2002) evaluated the same mixture of GOS and FOS at levels of 0.4 g/dL and 0.8 g/dL compared to control in term infants for 28 days. The number of fecal bifidobacteria and lactobacilli increased significantly in infants consuming both levels of the oligosaccharide mixture, and the stools were reported as softer with increasing dose. Schmelzle et al. fed a partially hydrolyzed protein formula supplemented with a combination of GOS and FOS at 0.8 g/dL to term infants for 12 weeks and documented an increase in bifidobacteria, softer stools, and adequate growth.

Euler et al. enrolled term infants in a 5-week prospective, randomized, cross-over study with a human-milk reference group. The infants received the same formula as reported in this trial, supplemented at 1.5 g/L or 3.0 g/L FOS. Bifidobacteria and lactobacilli counts were similar between the human milk and formula groups and did not change significantly with supplementation. Supplementation with FOS (3.0 g/L) resulted in more frequent and significantly softer stools.

All four studies reported an effect on stool pattern, which supports the data from our current study that oligosaccharides may decrease constipation. None of the trials demonstrated an effect on reducing pathogenic bacteria or a clinical benefit associated with increasing the fecal bifidobacteria.

In conclusion, the experimental cow's milk based formula supplemented with either 1.5 g/L FOS or 3.0 g/L FOS is safe and supports normal infant growth. The nutritional efficacy of either supplemented formula is similar to that of the control formula, and infants in all formula groups increased in size relative to reference ranges. Although the differences in the AE profiles of the formulas were not dramatic, the infants receiving the formula containing 3.0 g/L FOS had fewer AEs and fewer formula-related AEs, and in particular they experienced less constipation. This study contributes to the evaluation of FOS in term formula by monitoring growth and safety. Additional studies are needed to further examine the potential physiological benefits.

#### ACKNOWLEDGMENTS

The authors are grateful to the investigators participating in this multicenter trial: Gerald Bottenfield, Wesley Burks, Robert J. Cooper, Paul M. Darden, Barry L. Golembe, Fernando Gonzalez, Thomas Latiolais, Meredith Mahan, Dawn Martin, Aubrey McElroy, Mark A. Patterson, Bruce Pistorius, Keith S. Reisinger, Jack Seidel, Pamela Shaw, Warren Skaug, and Jon A. Vanderhoof. This study was funded by a grant from WYETH NUTRITION (Collegeville, PA, USA).

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