The Effects of Probiotics on Feeding Tolerance, Bowel Habits, and Gastrointestinal Motility in Preterm Newborns

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Objective To investigate the effect of dietary supplementation with a probiotic on feeding tolerance and gastrointestinal motility in healthy formula-fed preterm infants.

Study design Thirty preterm newborns were enrolled; 10 were exclusively breast-fed, and the remaining 20 were randomly assigned in a double-blind manner to receive either *Lactobacillus reuteri* ATCC 55730 (at dose of 1×10^8 colony forming units a day) or placebo for 30 days. Clinical symptoms of gastrointestinal function (regurgitation, vomiting, inconsolable crying, and evacuation) and physiological variables (gastric electrical activity and emptying) were recorded before and after the dietary intervention.

Results Body weight gains per day were similar for the 3 groups, and no adverse events were recorded. Newborns receiving probiotics showed a significant decrease in regurgitation and mean daily crying time and a larger number of stools compared with those given placebo. Gastric emptying rate was significantly increased, and fasting antral area was significantly reduced in both the newborns receiving *L. reuteri* and breast-fed newborns compared with placebo.

Conclusions Our results suggest a useful role for *L. reuteri* supplementation in improving feeding tolerance and gut function in formula-fed preterm newborns. *(J Pediatr 2008;152:801-6)*

The rationale for supplementing a formula-fed infant with probiotics is based on efforts to obtain the bifidogenic effect of breast-feeding. The larger number of bifidobacteria in the intestine of breast-fed infants has been associated with better health compared with formula-fed infants.[1] A growing number of studies involving formulas supplemented with probiotics and prebiotics have demonstrated that they can affect health outcome in both formula-fed and breast-fed infants.[2] The use of probiotics to promote food tolerance has also been proposed in preterm newborns.[3] However, to the best of our knowledge, the links between nutritional, clinical, and functional gastrointestinal variables, such as gastric electrical activity and motility, are lacking.

The intestinal microflora participates in the development and maintenance of gut sensory and motor functions by the release of bacterial substances, fermentation products and intestinal neuroendocrine factors, and through the effects of mediators released by the gastrointestinal immune system.[4,5] The end-products of colonic microflora fermentation (ie, the short-chain fatty acids [SCFAs] butyrate, acetate and propionate) may affect local and distant motor events via direct[4] and indirect (nervous) pathways, although the latter is still controversial.[5] The intestinal microflora is also essential for the normal development of the gut-associated lymphoid tissue.[6] Mediators released by immune cells are known to modulate various digestive functions, many of which involve the enteric nervous system,[7,8] the enteric smooth muscles, and interstitial cells of Cajal (ICC).[9]

Cutaneous electrogastrography (EGG)[10] is a reliable method for recording gastrointestinal motility. EGG studies in newborns[11-14] have demonstrated the absence of normal slow waves at birth and a maturation process that may or may not be modulated by enteral feeding. Normal gastric electrical activity and gastric emptying is detectable from 34 weeks gestational age, after which the pattern is similar to that of full-term

ANOVA	Analysis of variance	ICC	Interstitial cells of Cajal
DF	Dominant frequency	*L. reuteri*	*Lactobacillus reuteri* (ATCC 55730)
DFIC	Instability coefficient	NEC	Necrotizing enterocolitis
EGG	Cutaneous electrogastrography	SCFA	Short-chain fatty acid
GE	Gastric emptying		

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infants. Gastric emptying (GE) can be assessed by ultrasonography, a noninvasive technique particularly suitable for young patients.[15] We hypothesized that giving the probiotic *Lactobacillus reuteri* (ATCC 55730) (*L. reuteri*) to a preterm, formula-fed infant would improve feeding intolerance, bowel habit, and gastrointestinal motility patterns.

METHODS

Subjects and Protocol

The study was performed in the Neonatology Section of the Department of Pediatrics at the University of Bari. Between January and September 2006, 30 healthy appropriate for gestational age preterm infants, with normal Apgar scores, were enrolled on days 3 to 5 of life in this double-blind, controlled study. Newborns with respiratory distress, congenital malformation, inborn errors of metabolism, or proven sepsis or infection were excluded. Ten newborns were exclusively breast-fed, and the remaining 20 were randomly assigned to receive either *L. reuteri* at dose of 1×10^8 colony forming units (CFU) per day, delivered in an oil formulation (BioGaia AB, Sweden; 5 drops per day) or an indistinguishable placebo formulation for 30 days. The 20 randomized preterm newborns were all exclusively bottle-fed with the same standard formula throughout the intervention period. The daily formula intake was approximately 30 mL/kg/d at baseline and 180 mL/kg/d at the end of the study. The intake of breast milk in the breast-fed babies was calculated as the increase in the infant's weight after feeding. Written informed consent was obtained from the parents, and the institutional ethics committee at the Policlinico Università di Bari approved the study.

Symptom Evaluation

During hospital stay, the number of episodes per day of regurgitation (defined as the passage of refluxed gastric contents into the oral pharynx), vomiting (defined as the expulsion of the refluxed gastric contents from the mouth, i.e. feeding tolerance), inconsolable crying episodes (minutes per day as already described in literature[16]), and the number of evacuations per day (bowel habits) were recorded by the nurses. On discharge from the ward, parents were given written information about the study and were asked to record the same symptoms and any observations of adverse effects, by means of a structured diary. To aid the uniform documentation of crying times and to confirm that the infants were given the study products correctly, one of the investigators was always available by telephone to help parents.

Assessment of Gastric Electrical Activity

Gastric electrical activity was recorded on day 4 (time 0) and day 35 after birth. After overnight fasting, the EGG recordings were performed with portable equipment before and 120 minutes after a meal. Two silver-silver chloride bipolar electrodes (Clear Trace, ConMed, Utica, NY) were placed on the cleaned abdominal surface overlying the antropyloric axis to obtain the best signal-noise ratio. The reference electrode was placed to form an equilateral triangle.[14] Electrogastrography was performed with a portable EGG recorder (UPS 2020, Medical Management Systems, MMS, The Netherlands). All recordings were made at a sampling frequency of 1 Hz and the internal high- and low-pass filters were set at 1.8 and 16 cycles/min, respectively. After recording, the electrogastrogram data were fed into a personal computer and analyzed by means of the built-in software. In addition to the analysis available with the UPS 2020, we used Redtech GiPC software to perform further EGG data filtering and analysis. The following variables were evaluated for each subject:

Mean frequency of the EGG. The dominant frequency (DF) of the gastric peak was determined by the absolute peak value, and the mean frequency/power was computed by averaging the individual spectra.

Instability coefficient. This specifies the stability of the gastric electrical peak visible on the running spectra plot. It was calculated as the percentage ratio of the frequency standard deviation to the mean gastric frequency (DFIC).

The percentage of DF in the ranges defined as normal, bradygastric and tachygastric. A rhythmic gastric electrical activity ranging from 2.0 to 4.0 cycles/min was considered normal. Tachygastria was considered to be present when the running spectra had a dominant peak in the range 4.0-9.0 cycles/min, and bradygastria when the dominant peak was <2.0 cycles/min.

The power ratio. Because the absolute values of EGG power are influenced by several factors, (skin conductance, distance between the electrodes and the wall of the stomach, variable shape of the stomach, etc.), the EGG power can only be evaluated as the relative changes observed. The power ratio is the ratio of postprandial to fasting EGG power values.

The EGG signal was visually inspected to verify that no artifacts were present in any recording period. Periods containing these motion artifacts were deleted before computer analysis. EGG variables were obtained by means of running spectral analysis. This is currently the method most commonly used to analyze the EGG, and since Van der Schee et al[17] introduced the running spectral analysis in EGG, it has also been possible to analyze the frequency and amplitude changes over time. With this procedure, using a fast Fourier transform (FFT) the frequency components of 256 sec epochs of EGG signal are calculated, overlapped by 75%, and displayed as a 3-dimensional frequency plot.

Assessment of Gastric Emptying

Gastric emptying was recorded on day 4 (time 0) and day 35 after birth. The ultrasound gastric emptying examinations were always performed by the same investigator using a real-time apparatus (Image Point HX; Hewlett Packard